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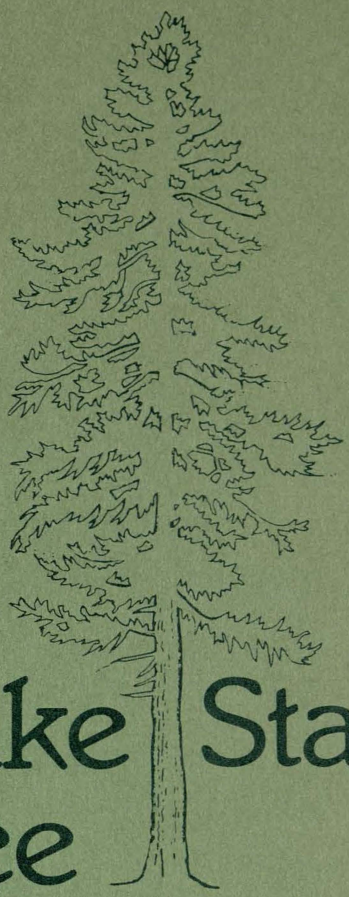

PROCEEDINGS OF THE

Thirteenth Lake States Forest Tree Improvement Conference

AUGUST 17-18, 1977

University of Minnesota
St. Paul, MN

orth Central Forest Experiment Station
orest Service, U.S. Department of Agriculture

North Central Forest Experiment Station
Robert A. Hann, Director
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ACKNOWLEDGMENTS

Thirteenth Lake States Forest Tree Improvement Conference

The Thirteenth Lake States Tree Improvement Conference was held at the University of Minnesota, St. Paul/Minneapolis, August 21-23, 1977. The Lake States Forest Tree Improvement Committee want to express its gratitude to the University staff for an excellent meeting.

ANNOUNCEMENT

LAKE STATES and CENTRAL STATES FOREST TREE IMPROVEMENT COMMITTEES TO MERGE

To reduce the number of meetings, to provide larger, better attended meetings and in order to facilitate the publication of proceedings the Executive Committees of the Lake States and Central State Organizations have decided to merge. The new organization will be known as

NORTH CENTRAL TREE IMPROVEMENT ASSOCIATION

The merger will be finalized at the FIRST NCTIA MEETING to be held at the University of Wisconsin, August 21-23, 1979.

The LAKE STATES FOREST TREE IMPROVEMENT COMMITTEE was organized at Eagle River, Wisconsin in March 1953. Through 1977 the Committee conducted the following 13 Tree Improvement Conferences:

Lake States Forest Genetics Conference, Eagle River, Wis., March 31-April 1, 1953.

Proceedings, Lake States Forest Genetics Conference, March 31-April 1, 1953. Lake States Forest Expt. Stn, Misc. Rpt. 22, 83 pp. (Processed) 1953.

Second Lake States Forest Tree Improvement Conference, Wisconsin Rapids, Wis., August 30-31, 1955.

Proceedings, Lake States Forest Tree Improvement Conference, August 30-31, 1955. Lake States Forest Expt. Stn., Misc. Rpt. 40, 108 pp., illus. (Processed) 1955.

Third Lake States Forest Tree Improvement Conference, Grand Rapids, Minnesota, September 17-18, 1957.

Proceedings, 3rd Lake States Forest Tree Improvement Conference, September 17-18, 1957. Lake States Forest Expt. Stn., Stn. Pap. 58, 87 pp., illus. (Processed) 1958.

Fourth Lake States Forest Tree Improvement Conference, Ann Arbor, Michigan, October 6-7, 1959.

Proceedings, 4th Lake States Forest Tree Improvement Conference, Lake States Forest Expt. Stn., USDA, Forest Service Station Rpt. 81. 60 p. 1960.

Fifth Lake States Forest Tree Improvement Conference, Rhinelander, Wisconsin, September 19-20, 1961.

Proceedings of the 5th Lake States Forest Tree Improvement Conference, Lake States Forest Expt. Stn., USDA, Forest Service Station Pap. 48, 42 p. April 1962.

Sixth Lake States Forest Tree Imprvoement Conference, Grand Rapids, Minnesota, September 8-9, 1963.

Proceedings of the 6th Lake States Forest Tree Improvement Conference, Lake States Forest Expt. Stn., USDA, Forest Service. 90 p. August 1964.

Seventh Lake States Forest Tree Improvement Conference held jointly with Second Genetics Workshop of the Society of American Foresters, Hickory Corners, Michigan, October 21-23, 1965.

Joint Proceedings 2nd Genetics Workshop of the Soc. of American Foresters and the 7th Lake States Forest Tree Improvement Conference, USDA Forest Service Research Paper NC-6. 110 p. 1966.

Eighth Lake States Forest Tree Improvement Conference, Madison, Wisconsin, September 12-13, 1967.

Proceedings of the 8th Lake States Forest Tree Improvement Conference. USDA, Forest Service Research Paper NC-23, 60 p. 1968.

Ninth Lake States Forest Tree Improvement Conference, Grand Rapids, Minnesota, August 22-23, 1969.

Proceedings of the 9th Lake States Forest Tree Improvement Conference. USDA, Forest Service Research Paper NC-47. 34 p. 1970.

Tenth Lake States Forest Tree Improvement Conference held jointly with Seventh Central States forest Tree Improvement Conference, Madison, Wisconsin, September 22-24, 1971.

Joint Proceedings of the 10th Lake States Forest Tree Improvement Conference and the 7th Central States Forest Tree Improvement Conference. USDA, Forest Service General Technical Report NC-3, 61 p. 1973.

Eleventh Lake States Forest Tree Improvement Conference, Hickory Corners, Michigan, October 10-12, 1973.

This meeting was entirely devoted to field trips. No Proceedings were published.

Twelfth Lake States Forest Tree Improvement Conference held jointly with the Canadian Tree Improvement Association, Petawawa Forest Experiment Station, Chalk River, Ontario, August 18-22, 1975.

Proceedings of the 12th Lake States Forest Tree Improvement Conference. USDA, Forest Service General Technical Report NC-26. 206 p. 1976.

Thirteenth Lake States Forest Tree Improvement Conference, St. Paul/ Minneapolis, Minnesota, August 17-18, 1977.

The 12 Proceedings were all published by the North Central Forest Experiment Station, USDA, Forest Service (formerly Lake States Forest Experiment Station). The Experiment Station provided technical review and editorial services and covered all expenses connected with publications.

The Lake States Forest Tree Improvement Committee wishes to thank the North Central Forest Experiment Station for this great contribution to the Committees work and to Tree Improvement and Genetics Research in the Lake States.

Hans Nienstaedt
Executive Secretary
Lake States Forest Tree Improvement Committee

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PROMOTION OF FLOWERING IN THE PINACEAE [1] BY HORMONES -- A REALITY

Richard P. Pharis [2]

ABSTRACT.--The effectiveness of gibberellins as promotors of flowering in Cupressaceae and Taxodiaceae is historically reviewed, emphasizing that these groups of plants respond to polar gibberellins and produce sexual structures after exogenous application of GA_3. Species in Pinaceae, on the other hand, can be brought into flowering with non-polar gibberellin treatments. Admixtures of gibberellins applied with auxin and stimulatory cultural treatments such as strangulation, nitrate fertilization, root pruning, or drought stress are particularly successful. Each species, age, even each clone and each site may require highly "specific" treatments in forms of dosage level, frequency of application, hormone mixture, and adjunct cultural treatment.

In this paper I will discuss the effects of certain of the gibberellins on flowering of young seedlings, grafted ramets, and older trees in the Pinaceae. I will also give you some history of the work that went into obtaining the results. I do this for two reasons: One is to show you that the induction, or promotion of flowering in Pinaceae is not a simple procedure; gibberellins are not a patent medicine. You can't just walk through a seed orchard and spray the hormones on the trees and stand back and wait for the flowering. It takes a knowledge of the period of "sexual" differentiation, and may require other cultural treatments in addition to the hormones. Results from a given dosage rate, or application

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[1]This paper draws heavily on individual and collaborative work by the following: A. Dunberg, J. Glenn, G. Kiss, C. G. Kuo, W. Morf, J. Owens, R. M. Rauter, G. Sweet, R. Wample, and N. Wheeler.

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STRUCTURES OF GIBBERELLINS

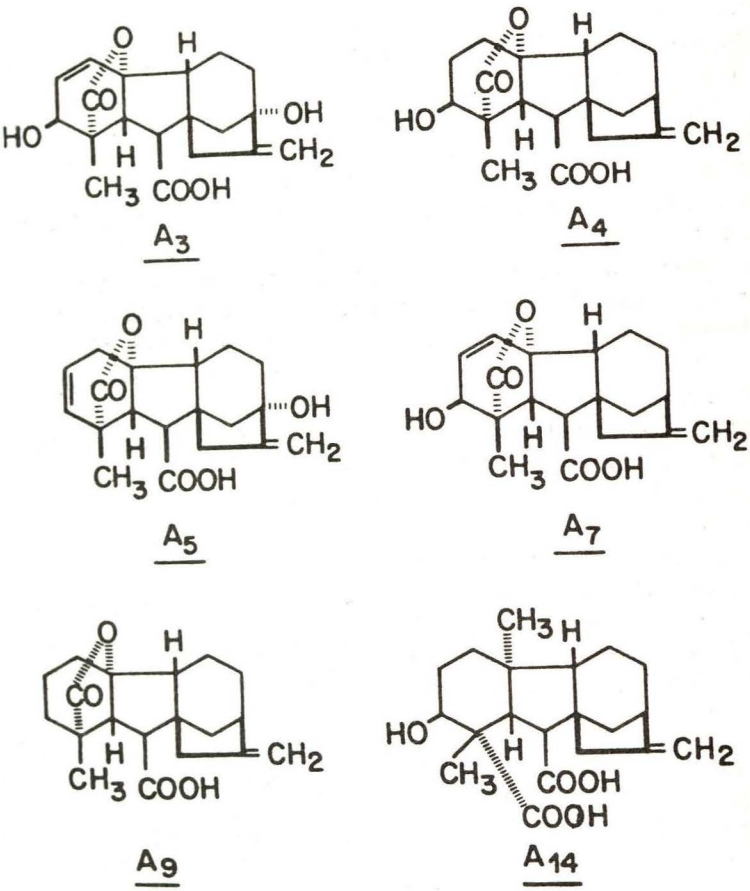

Fig. 1. Structures of 6 gibberellins (GAs) known to promote flowering in conifers. Interconversion occurs between GAs from a less-oxidized state (i.e., GA_4, GA_5, GA_7, GA_9, and GA_{14}) to a more oxidized state (i.e., GA_3) in both the fungus, (Gibberella fujikuroi (Saw.) Wollenw. and higher plants (see reviews by West, 1973, and MacMillan, 1974). All except GA_{14} are known to be native to higher plants and GA_3, GA_4, GA_7, GA_9, and GA_{14} can be produced in quantity by G. fujikuroi. Pathways of interconversion in the fungus are well-known, but are only now being ascertained in higher plants. Figure taken from Pharis et al. (1976).

frequency may vary from year to year and from clone to clone. Each species may have specific requirements with regard to type, mixture, and dosage of hormone, timing of treatment, frequency of application, and best type of additional cultural treatment.

A bit of history will also show that the hormones in question are discrete entities, not just a "class" of chemical compounds called gibberellins of which 51 are currently known (for structure, see review by Pharis and Kuo 1977).

Those of you who are not chemists or physiologists probably tend to think of auxins, cytokinins, and gibberellins as classes of hormones, individual compounds within a class having the same effect. Especially with gibberellins, this is not the case. Different gibberellins have different effects, and it is probable that plants regulate growth and differentiation by controlling the level and type of endogenous gibberellin (and other hormones).

The structures of six gibberellins known to promote flowering in conifers are shown in Fig. 1: Notice the presence or absence of the hydroxyl (-OH) group. The gibberellin that you can buy in the horticultural stores to put on your begonias is GA_3; it has two hydroxyl groups on different rings. Another gibberellin, GA_9, has no hydroxyl groups whatsoever. Gibberellins A_4 and A_7 (as a mixture) can be purchased in quantity from Plant Protection (ICI) Ltd., Fernhurt, Haslemere, Surrey, England. Gibberellin A_9 is available in small quantities only for research purposes. The other gibberellins shown are not currently available.

Gibberellins are either polar or non-polar. The polar gibberellins are those with two or more hydroxyl groups. The non-polar gibberellins have no (i.e., GA_9) or only one hydroxyl (i.e., GA_4, GA_5, or GA_7) group. Hydroxyl groups are indications of the state of oxidation. Gibberellins, as potent hormones, may be "gotten rid of" by the plant by oxidative metabolism. When you administer a gibberellin to a plant, one of its first reactions is to hydroxylate! The oxidative sequence goes from no or few hydroxyls to many hydroxyls--from a low state of oxidation to a high state of oxidation.

Secondly, the plant may begin to conjugate the gibberellins with glucose and perhaps other sugars (or sulphur, or propyl or peptidyl groups). I think that many of our past studies on gibberellins in conifers have been failures, because conifers turn out to be extremely adept in getting rid of gibberellins (c.f. ^3H-GA_4 metabolism in Douglas-fir; Wample *et al.* 1975).

Tree breeders need "sexy" trees for breeding and seed production--trees with lots of cones and lots of seeds. An example of this is shown for some ramets in a Douglas-fir seed orchard on Vancouver

Island. These grafted ramets flowered profusely; there was only one trouble, they died shortly thereafter from graft incompatibility. This profuse, or early flowering resulting from stress or trauma is a most important reaction exhibited by most woody perennial plants. It (a) gives us a tool by which we may study the physiology and biochemistry of flowering, and (b) offers us an adjunct treatment by which we may "amplify" hormone-induced flowering.

The history of gibberellins in the flowering of conifers began with the Japanese work (Y. Kato and H. Hashizume; see review by Pharis and Kuo 1977) on species of Cupressaceae and Taxodiaceae in 1956 and 1957. As examples of the high efficacy of GA_3 within these families, a seedling of Thuja plicata only 4 cm high can be made to produce staminate strobili; an ovulate strobilus was produced on a 3-month-old Sequoiadendron seedling; and for Cupressus arizonica, a 12-month-old plant sprayed with GA_3 over a period of several months, produced 8,007 strobili out of 8,090 meristems. These conifers, by the way, are nice tools for the physiologist: on just one plant there can be 8,000 to 10,000 meristems, most of which can be transformed quickly from the vegetative to reproductive state.

In addition to being "sexy" hormones, gibberellins in conifers do a lot of other things. As endogenous hormones, they affect stem elongation and diameter growth, and exogenous applications of various gibberellins will increase internode elongation, affect the apical form and apical dominance (enhance or decrease, depending upon the species), and increase diameter growth and shoot dry weight (see review by Pharis (1976a) and Pharis and Kuo (1977).

Thus, we are looking at hormones that are native to conifers, and do a lot of things besides making conifers "sexy". In fact, they appear to do most, if not all, of the vegetative growth "jobs" before doing any "reproductive work," One can perhaps generalize that gibberellins are "sexy hormones" only after they have accomplished certain vegetative growth processes; only after they build up to a certain threshold concentration do they cause sexual differentation. I propose that in the juvenile plant the gibberellins are preferentially used for vegetative purposes, sexual maturity occurring when there are enough gibberellins left over. This will generally occur on the odd meristem here and there, and only in the case of stress will a young conifer flower profusely. In each case--normal maturation, or stress-induced flowering--I propose that the flowering is caused by "left-over" endogenous gibberellins that have not been used or cannot be used for vegetative growth.

We knew from the early work of the Japanese, and from our own work, that we could readily get Cupressaceae and Taxodiaceae to flower with gibberellin A_3. We also knew that other gibberellins were effective for these two families. But Pinaceae species would not flower in response to gibberellin A_3. Furthermore, certain stress, or cultural treatments that are successful on Taxodiaceae and Cupressaceae work well in Pinaceae (see Literature Cited in Pharis (1976)), thus lending credance to a "common cause". Now, what are some of the possible reasons for this conundrum?

First, the growth habit or a meristem production habit in Cupressaceae and Taxodiaceae species is indeterminant. In other words, about every 15 to 30 days a new meristem is produced. Thus, 10 to 20 times a year each actively growing meristem has a "chance" to initiate a reproductive structure from a previously vegetative meristem (see Owens and Pharis 1967, 1972).

Thus, in the Cupressaceae and Taxodiaceae, we have access to large numbers of meristems that are continually turning over and making themselves available to possible hormonal effects resulting from exogenous hormone application. In most Pinaceae species, on the other hand, meristems can only be induced once a year, and the period of possible induction takes place during a few weeks. During these few weeks, then, its lateral primordia are trying to "decide" whether they will be lateral vegetative, lateral "latent", will abort, or whether they will become reproductive. This, then, is the time one has to hit them with the message, the hormonal signal. Perhaps many of our past experiments with hormone application missed the "differentiative" stage. In essence, an effector treatment that will induce sexual differentiation in the Pinaceae is doing so by acting upon pre-existing, undifferentiated primordia (Owens and Molder 1975), unlike Cupressaceae and Taxodiaceae whose reproductive structures are initiated from previously vegetative meristems.

In addition to the wrong timing in our experiments with Pinaceae species, we may have had the wrong gibberellin (i.e., perhaps GA_3 was a "flowering" gibberellin in Cupressaceae and Taxodiaceae, but not in Pinaceae). We therefore went back and looked at situations that would allow us to determine whether we had the right or wrong gibberellins.

We examined cultural treatments, which often (but not always), when used as the sole treatment, cause flowering. There are a number of these: starvation, nitrogen or phosphorus fertilization, girdling or strangulation, root pruning, drought stress, nitrate fertilization (in Douglas-fir, at least) are good examples. In three separate tests we applied certain of these cultural treatments to trees and then determined the endogenous gibberellins of control versus treated trees.

In *Cupressus arizonica* we repeated (Kuo 1973) the classic work of Lyr and Hoffman (1964), inducing both male and female flowering on *Cryptomeria japonica* by nitrogen starvation. In Douglas-fir, flowering can be promoted with either drought stress or calcium nitrate fertilization (Ebell 1967, 1972). On a low nitrogen site, Dr. Ebel fertilized a stand of Douglas-fir trees with calcium nitrate. Buds were sampled from treated and untreated trees, freeze-dried, extracted, analyzed for endogenous gibberellins, and the results correlated with induced flowering. Finally, Douglas-fir seedlings were subjected to drought stress.

In each experiment trees given the "flower-inducing" cultural treatment had large amounts of non-polar gibberellins compared with the controls. Conversely, the later fractions, the GA_3-like fractions and other more polar "bioassay active" fractions, were lower in trees that flowered and higher in the control trees that stayed vegetative.

So, three different cultural treatments produced similar results. Now, remember that trees that build up non-polar gibberellins are possibly trees that are not able to metabolize (i.e., hydroxylate) these non-polar gibberellins to more polar gibberellins. Thus, there may be a blockage of oxidative metabolism (or else increased synthesis over and above the ability of the tree to readily interconvert the extra quantities).

With these results in mind, we went into the seed orchards with an increased spectrum of gibberellins. We applied polar GA_3 (which we knew did not work from earlier experiments) as well as a number of the non-polar gibberellins (GA_4, GA_7, GA_9, GA_5) to grafts and seedlings of Douglas-fir and seedlings of lodgepole pine. The results were spectacular, to say the least (see appropriate figures and tables in Ross and Pharis (1976), Pharis *et al.* (1975), Pharis *et al.* (1976), Pharis 1975), Pharis and Kuo (1977)). Non-polar gibberellins, especially the $GA_{4/7}$ misture with GA_9, and a low concentration of the auxin, NAA, causes highly significant flowering--GA_3 is essentially ineffective! These earlier results have now been extended to a number of other *Pinaceae* species (see review by Pharis and Kuo 1977).

The strobili induced by the non-polar gibberellins can be manipulated with regard to sex. In Douglas-fir, high auxin concentrations promote male cone differentiation, low auxin increases females (just the opposite from what one would expect from work with herbaceous plants). We don't yet understand why this is the case, but for Douglas-fir, at least, work by Dr. S. D. Ross of Weyerhaeuser has confirmed this phenomenon in many experiments.

Thus, in the Pinaceae we have only a single period, perhaps 6 to 10 weeks, to "tell" previously undetermined buds what they are to become, whether to be male or female, vegetative, latent, or to abort. An effective treatment during this 6- to-10-week period may require that a certain threshold concentration of hormone be maintained during the entire period, and further, that photosynthate and other nutrients be readily available, not only during the 6 to 10 weeks of early differentiation, but subsequently as well. If any of these "promotive factors" is not maintained at an appropriate level, sexual differentiation ceases and abortion, or increased "latency" may occur.

Now, what about juvenility per se? Initially, our results were best on saplings or grafted ramets, and nil on young seedlings. Some of Dr. Ross's early work with 4-year-old seedlings showed only a non-significant" tendency" for $GA_{4/7}$ mixture to promote flowering. But, when he used cultural treatments in addition to the $GA_{4/7}$ mixture, flowering was appreciably enhanced on young seedlings.

If one wants to get young seedlings, or recalcitrant grafted clones to flower, I recommend most strongly the use of a $GA_{4/7}$ mixture with an auxin treatment and an additional cultural treatment (such as modest water stress, nitrate fertilization, or non-destructive cambial girdling). I further recommend future location of all seed orchards in warm, sunny locations (especially during early differentiation) where modest water stress in spring can be alleviated by irrigation in summer.

One of our more recent studies into just how a cultural treatment may "assist" the $GA_{4/7}$, or may cause flowering on its own, was done by Dr. A. Dunberg, at the Royal College of Forestry in Umeå, Sweden on Picea abies. Grafted ramets of Picea abies can often be made to flower by surrounding them with plastic on a light frame. Dr. Dunberg also got flowering with the non-polar gibberellins--the $GA_{4/7}$ and GA_9 treatment being most effective. Thus, as with Douglas-fir, a non-hormonal "flower-inducing" cultural treatment existed for P. abies, and P. abies also responded to treatment with non-polar gibberellins.

Trees surrounded by plastic were given a radioactive gibberellin (3H-GA_4) of very high specific activity (i.e., the actual amount of GA_4 was so low as to be metabolized by the tree as if it were a "native" gibberellin). The GA_4 was not enough for the tree to "see" physiologically, but was radioactive enough for us to see in terms of how the tree metabolized it. After 48 to 96 hours of incubation in the field, extracts of control and "plastic-treated" branches were made and analyzed by gas chromatography-radiochromatogram scanning. Under conditions that cause flowering (i.e., plastic on frames), metabolism of 3H-GA_4 to more polar gibberellins was depressed,

relative to metabolism in control branches. Thus, a cultural treatment that causes flowering, results in a build-up of the non-polar GA_4, relative to non-flowering control branches--a situation analogous to the work on endogenous gibberellins of Doulas-fir and Cupressus arizonica given flowering promotive cultural treatments!

Some other examples of flowering promotion in Pinaceae species are as follows:

Pinus contorta grafted ramets. The $GA_{4/7}$ mixture dripped onto the bud; Prince George, B.C., N. Wheeler, B.C. Forest Service, Research Division. For the treated terminal shoot, frequency of flowering: control-33%; low $GA_{4/7}$--54%; high $GA_{4/7}$--42%. Notice, the supraoptimal effect of $GA_{4/7}$. If we look at the number of females: 0.67--control; 1.79--low $GA_{4/7}$; 1.17--high $GA_{4/7}$. Again, the supraoptimal effect. But, if we look now at females on lateral branches adjacent to the treated shoots: control--17%; low $GA_{4/7}$--21%, high $GA_{4/7}$--25. If we look at number of females on adjacent lateral branches: 0.17--control; 0.71--low $GA_{4/7}$; 0.67--high $GA_{4/7}$. If we look at all other branches on the ramets, flowering frequency is control--25%; low $GA_{4/7}$--29%; high $GA_{4/7}$--38%. Number of flowers for "all other branches" of the ramet: 1.08--control; 1.04--low $GA_{4/7}$; 1.54-$GA_{4/7}$. And, if for this experiment we pull a "sexy" clone out; (SK5) clone comes out: For treated terminals, control--11%, low $GA_{4/7}$--27%, high $GA_{4/7}$--37%; for number of females on the terminals, control--0.18, low $GA_{4/7}$--0.55, high $GA_{4/7}$--0.82.

Picea glauca grafted ramets. Work by G. Kiss, B.C. Forest Service, Research Division, Prince George, B.C. shows a female flowering frequency of: control--0 out of 48; low $GA_{4/7}$--7 out of 48 females; high $GA_{4/7}$--5 out of 48. For number of female strobili (total): control--0.0; low $GA_{4/7}$--15.0; high $GA_{4/7}$--14.0. But, because of the adjunct girdling treatment many branches were lost to snow breakage. For example, control without girdling--0 of 48 branches lost; control with girdling--15 out of 48; low $GA_{4/7}$--15 out of 48; high $GA_{4/7}$--21 out of 48 branches lost. However, although we lost almost half of the branches in the high $GA_{4/7}$ treatment, we still got reasonable flowering (i.e., 14 females).

Similarly good results on P. glauca were obtained by R. M. Rauter (using GA_4 on grafted ramets) in both 1976 and 1977, and in an experiment on 55-year-old P. glauca trees. $GA_{4/7}$ plus auxin plus girdling proved to be an effective treatment, control flowering being essentially zero.

In summary, flowering promotion in many Pinaceae species is now a reality, using certain of the non-polar gibberellins, especially in admixture, and with an auxin and adjunct "cultural"treatments. However, each species, each age, each clone, and each site (i.e., geographic location) may have to have highly "specific" treatments (in terms of dosage level, frequency of application, hormone mixtures, and adjunct cultural treatments) developed to insure success. Results thus far lead me to strongly recommend the "hormonal" approach for the breeder, but it is still too early to make such a recommendation to the seed orchard manager--much additional research work is required in this latter area.

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LONGLEAF PINE FLOWERING IN RESPONSE TO NITROGEN FERTILIZATION, BRANCH GIRDLING, GROWTH SUBSTANCES, AND CULTIVATION

R. C. Hare, E. B. Snyder, and R. C. Schmidtling[1]

ABSTRACT.--Biweekly applications of 400 µg $GA_{4/7}$ plus 25 µg NAA per bud from June 1 to August 10 promoted male and female flowering in longleaf pine (Pinus palustris Mill.), especially when combined with partial branch girdling and NH_4NO_3 fertilization. Fertilization was the most effective single factor, and girdling was second. Cultivation had no significant effect on flowering. Vegetative bud growth was doubled by GA-NAA, independent of fertilizer stimulation.

Poor pollen production has seriously hindered improvement breeding in longleaf pine. Certain cultural treatments (nitrogen fertilization, cultivation, subsoiling) and branch treatments (partial girdling, gibberellins) increase flowering in conifers. Varnell (1970) found that partial branch girdling increased eightfold the number of longleaf pine branch tips bearing male flowers, but did not increase female flowering. With loblolly pine (P. taeda L.) nitrogen fertilization promotes female flowering (Schmidtling 1974, van Buijtenen 1966). However, van Buijtenen reported that this was accompanied by a decrease in male flowering, and he also found that cultivation increased both male and female flowering.

Gibberellins (GAs) have enhanced flowering in many conifers (Pharis 1974, 1975, 1976). GA_3 is effective except in Pinaceae species, which require a less polar gibberellin like $GA_{4/7}$. Biweekly bud applications of 400 µg $GA_{4/7}$ combined with partial branch girdling promoted flowering in loblolly pine. Low levels of naphthaleneacetic acid (NAA) combined with $GA_{4/7}$ further improved flowering.

Our experiment tested effects of partial branch girdling, GA-NAA bud application, NH_4NO_3 fertilization, and shallow cultivation on the percentage of buds with male and female flowers. An attempt was also made to evaluate the effects of these treatments on the number of flowers per flowering bud.

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MATERIALS AND METHODS

Twenty 13-year-old longleaf pines with poor flowering histories were selected from a thinned south Mississippi plantation which was beginning to flower. Average diameter at breast height was 18 cm.

Four cultural treatments (control, fertilized, disked, and fertilized plus disked) were randomly assigned in each of five blocks containing four trees each. In late May, 900 g NH_4NO_3 were distributed evenly among six 30 cm deep holes around the drip lines of the designated trees. Trees selected for soil treatments were far enough apart to avoid cross-over effects. Light disking beneath the crown was done in late May.

Eight branches were selected on each tree--four in the lower crown and four in the mid- to upper crown (hereafter called upper). Four branch treatments (control, partial girdle, GA-NAA, and partial girdle plus GA-NAA) were randomly assigned at each crown level. Branches were girdled in late May 1976 by removing two staggered strips of bark and cambium. Strips were 1 cm apart, 1 cm wide, and covered 3/4 of the branch circumference. From June 1 to August 10 growth substances were applied biweekly to each bud in dosages of 400 µg $GA_{4/7}$ and 25 µg NAA in 0.1 ml 50% ethanol.

In February 1977, each bud was measured for length, and male and female flowers were counted. Data were then calculated on a branch basis for average bud length, percentage of buds with male flowers, percentage with female flowers, and average number of male and female flowers per flowering bud.

The statistical design was a split-split plot with five replicates: whole plots were soil treatments, first order subplots were crown levels, second order subplots were branch treatments. Analysis of variance was used to test for significance at the 0.05 level of probability.

RESULTS AND DISCUSSION

Bud length increased with nitrogen fertilization and doubled with GA-NAA treatments (fig. 1). Disking had no effect and girdling reduced bud growth. Girdling plus GA-NAA increased bud length less than did GA-NAA alone. For bud growth, there were no significant soil/branch or soil/crown level interactions. Upper crown buds averaged more growth than lower crown buds.

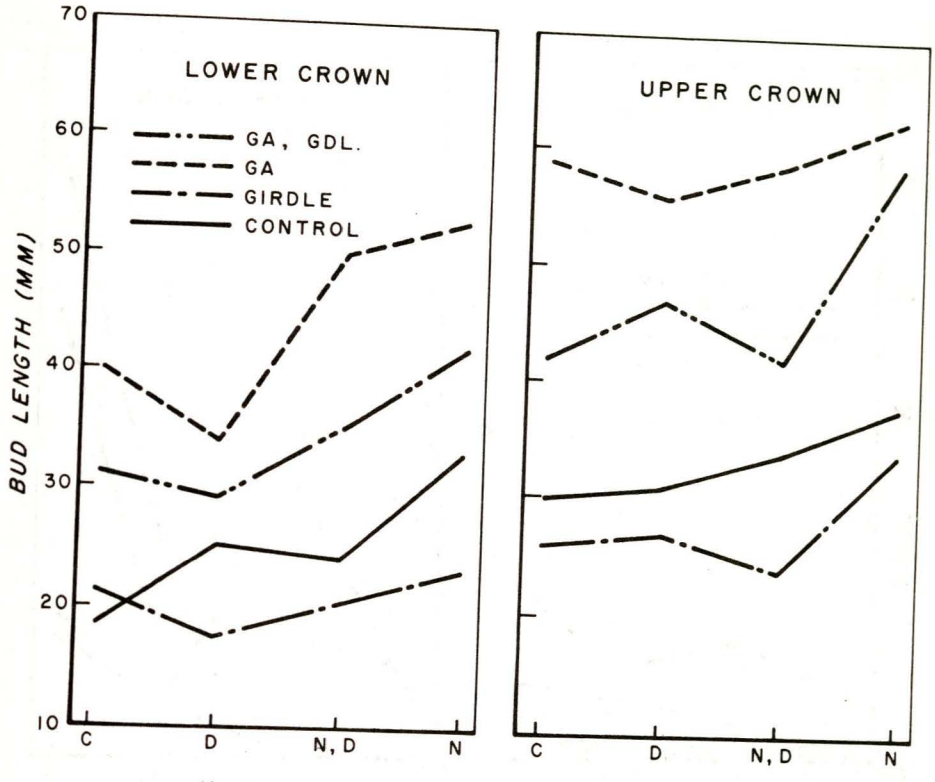

Figure 1.--Effect of soil and branch treatments on average bud length. Soil treatments were control (C), disking (D), NH_4NO_3 fertilization plus disking (N,D), and NH_4NO_3 fertilization alone (N). Branch treatments were control, girdle, $GA_{4/7}$ - NAA (GA), and girdle plus $GA_{4/7}$-NAA (GA,GDL).

Nitrogen generally promoted both male and female flowering, but there was no significant effect from disking nor nitrogen plus disking (figs. 2 and 3). In fact, the combination of disking and nitrogen usually produced fewer flowers than either treatment alone.

All three branch treatments promoted flowering. At both crown levels female flowering was promoted best by GA-NAA plus girdle (fig. 2). Combined with nitrogen fertilization this treatment gave 50% female flowering in the upper crown, compared with no flowering on the control. For male flowering in the upper crown the combined branch treatment was less effective than girdling alone (fig. 3), which produced 60% flowering on fertilized trees. In the lower crown the three branch treatments were about equally effective for male flowering.

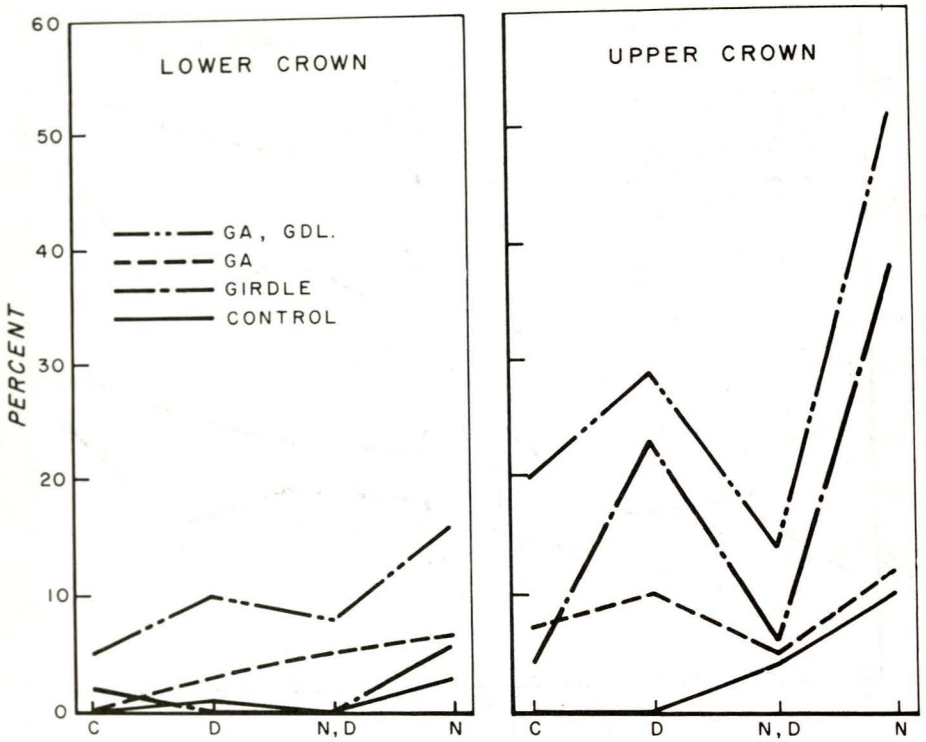

Figure 2.--Effect of soil and branch treatments on percentage of buds with female flowers. Soil treatments were control (C), disking (D), NH_4NO_3 fertilization plus disking (N,D), and NH_4NO_3 fertilization alone (N). Branch treatments were control, girdle, $GA_{4/7}$-NAA (GA), and girdle plus $GA_{4/7}$-NAA (GA, GDL).

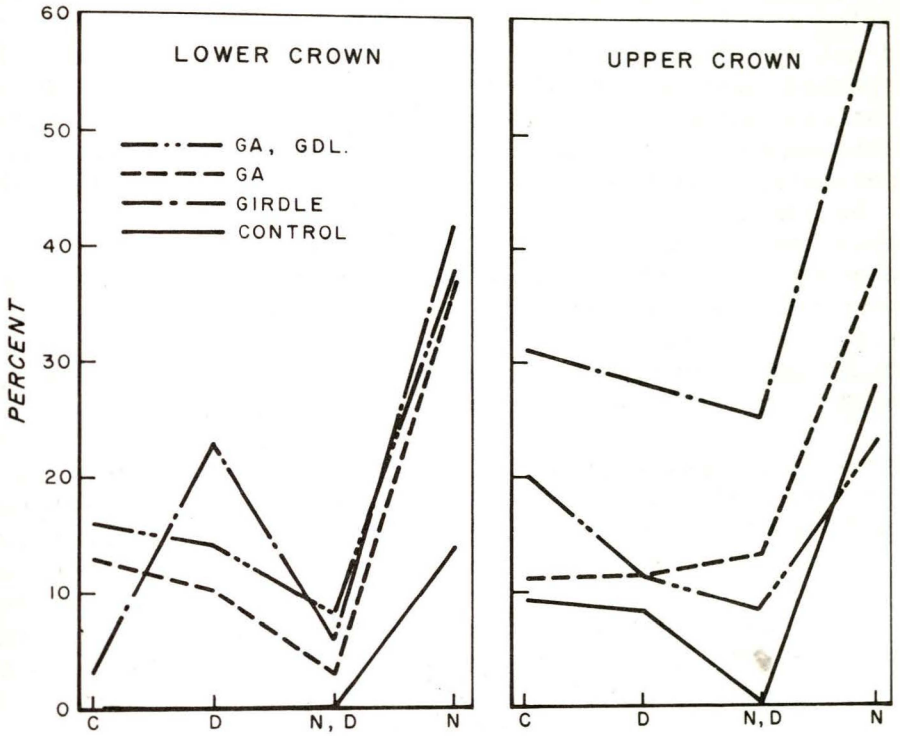

Figure 3.--Effect of soil and branch treatments on percentage of buds with male flowers. Soil treatments were control (C), disking (D), NH_4NO_3 fertilization plus disking (N,D), and NH_4NO_3 fertilization alone (N). Branch treatments were control, girdle, $GA_{4/7}$-NAA (GA), and girdle plus $GA_{4/7}$-NAA (GA,GDL).

Most of the literature on promoting pine flowering deals only with the percentage of branch tips having male or female flowers. More important is the total flower production, which depends not only on percentage of flowering buds but also on number of flowers per flowering bud. Unfortunately, treatment effects on flowers per flowering bud in this study could not be compared statistically because some branches had no flowers. It appeared, however, that branch treatments increased flowering percentage but decreased the average number of flowers per flowering bud, whereas nitrogen fertilization markedly increased both male and female flowering percentage and number of flowers per flowering bud. These observations might be explained if nitrogen is a limiting factor in flower initiation and development. Induction of more flowering buds by branch treatments may channel nitrogen for amino acid synthesis into these new sinks, thus limiting substrate per bud and allowing fewer flowers to form. Girdling may induce more buds to flower by keeping organic nitrogen compounds and carbohydrates from moving out of the branch via the phloem, but without nitrogen fertilization the number of flowers produced will be limited.

From this experiment we conclude that a combination of NH_4NO_3 fertilization, partial branch girdling, and bud treatment with $GA_{4/7}$ plus NAA most effectively promotes longleaf pine flowering. Cultivation is ineffective or even detrimental with fertilization. At present only fertilization appears to lend itself to an operational program in seed orchards. Girdling takes too much time and occasionally kills the branch. If GA-NAA could be applied by foliar spray the treatment would be practical, but GA appears to require tedious application to individual buds (Pharis *et al.* 1975).

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EFFICIENCY OF SEED PRODUCTION IN SOUTHERN PINE SEED ORCHARDS

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ABSTRACT.--Seed production in southern pine seed orchards can be evaluated by estimating the efficiency of four separate stages of cone, seed, and seedling development. Calculated values are: cone efficiency (CE), the ratio of mature cones to the initial flower crop; seed efficiency (SE), the ratio of filled seeds per cone to the seed potential; extraction efficiency (EE), the ratio of extracted seeds per cone to the total seeds; and germination efficiency (GE), the ratio of germinated seeds per cone to the filled seeds. The product of these four efficiency values is the overall seed orchard to nursery efficiency (SO-NE). With SO-NE, the orchard manager can compare the actual yield of seedlings produced from the orchard to the maximum biological potential of a given flower crop to produce viable seeds. The two approaches to increasing production of seed orchards are to increase flower production and to increase CE, SE, EE, or GE values. Computation of efficiency values pinpoints major types of seed losses and shows where corrective actions should be concentrated.

The tree improvement program in the South now has many thousand acres of established pine seed orchards. The seeds from these orchards contain the improved growth, wood quality, and pest resistance of a new generation of forest trees. To economically meet the demand for these seeds, it is important that each orchard produce seeds efficiently.

The reproductive process begins with initiation of flower primordia and ends with seedlings. In this paper, however, I discuss only the development and maturity of seed after the flowers have been formed. The primary stages of seed orchard production discussed are cone development, seed yield per cone, extraction of mature seeds, and germination of filled seeds.

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CONE DEVELOPMENT

Survival and normal development of a high percentage of cones is critical because loss of a cone means loss of its entire set of seeds. For a given flower crop in the seed orchard, cone development can be evaluated in terms of cone efficiency. This value is the ratio of the harvested cones to the original flower crop.

$$\text{Cone efficiency} = \frac{\text{Number of harvested cones}}{\text{Number of female flowers initiated}} \times 100\%.$$

The calculated cone efficiency value gives the overall survival of a flower crop but it does not identify specific causes of cone mortality. In shortleaf pine (Pinus echinata Mill.) studies in Virginia, the majority of mortality occurred during the spring and summer following pollination. The average cone efficiency for flower crops from 1963-1969 was 29 percent; 61 percent of the potential cone crop was lost in the first year and only 10 percent lost in the second year (Bramlett 1972). To evaluate seasonal losses, a periodic count is necessary. The Georgia Forestry Commission Seed Orchard has utilized a subsample of 10 tagged branches on randomly chosen sample trees. The cones on the tagged branches were counted a total of 10 times during the cone developmental period and flower and clone mortality were recorded (Godbee and others 1977). DeBarr and Barber (1975) charted the development of a flower crop and identified specific causes of cone mortality on life tables.

Since cone efficiency is the percent survival of an initial flower crop, the actual number of original flowers strongly influences cone efficiency. With a constant cone efficiency a greater number of flowers are lost as the size of the flower crop increases. Conversely, if the number of cones lost in each flower crop remains constant, then the cone efficiency decreases for small flower crops and increases for large flower crops. A further complication occurs when the previous cone crop influences the succeeding crop in terms of increasing mortality the year following a year with abundant flowering. This increase apparently is the result of an increase in insect populations from the preceding year. For example, cone efficiency of a natural stand of shortleaf pine was only 3 percent in 1964 as a result of a severe spring frost in 1963. The following year a large flower crop was produced with a correspondingly high cone efficiency of 65 percent. The flower production remained relatively high for the next two years, 1966 and 1967, but mortality in numbers of cones increased annually and the cone efficiency likewise decreased. Flower production was again low in 1968 but cone efficiency remained at 25 percent. Flower production increased to its highest level in 1969 and the projected cone crop (based on first-year survival) would give a cone efficiency of approximately 48 percent.

SEED YIELD PER CONE

Once the cones are mature and harvested, the seed yield per cone can be measured and compared to the capacity of the cone to produce seeds. Each pine cone contains a series of scales spirally arranged on a central axis. The cone scales can be counted and classified as fertile or infertile in a cone analysis technique developed by Bramlett and others (1977). Average values for total number of scales on sampled cones from 4 southern pines were 87 for shortleaf, 111 for Virginia (P. virginiana Mill.), 135 for loblolly (P. taeda L.), and 149 for slash pine (P. elliottii Engelm.) (Bramlett 1974). The total number of scales per cone, however, is not an accurate measure of the capacity of the cone to produce seeds. To quantify the seed production capacity, only the fertile or seed bearing scales should be counted (Lyons 1956). The fertile scales occur in the upper one-half to two-thirds of the cone and are distinguished from the lower, infertile scales by the presence of functional ovules or seeds. Also, fertile scales are wider at the base and in general larger than infertile scales.

Each fertile scale is capable of producing two seeds. Thus, for each cone, seed potential is:

$$\text{Seed potential} = 2 \times \text{number of fertile scales.}$$

Seed potential is the maximum number of seeds that the cone is biologically capable of producing. The seed potential observed in sample cones averaged 170 for slash pine, 155 for loblolly pine, 88 for Virginia pine, and 87 for shortleaf pine (Bramlett 1974).

Although the seed potential establishes the upper biological boundary for seed production from a given cone, the actual yield of filled seeds is the only product of value from the orchard. Seed efficiency is determined by comparing the yield of filled seeds per cone with the seed potential for that cone.

$$\text{Seed efficiency} = \frac{\text{Number of filled seed}}{\text{Seed potential}} \times 100\%.$$

Seed efficiency can be used to evaluate the seed performance of a given tree, clone, or seed orchard. For example, a sample of slash pine cones in 1972 yielded 28 filled seeds per cone. Since the seed potential was 170 seeds, the seed efficiency was 28/170 = 16 percent. Comparable values for loblolly, Virginia, and shortleaf pine were 24 percent, 19 percent, and 14 percent for the samples evaluated.

SEED LOSSES

Seed efficiency values ranging from 14 to 24 percent seemed disappointing, and it was important to examine the causes of seed failure in the remaining 86 to 76 percent of the potential seeds. The cone

analysis procedure was used to place all ovulates and seeds on the extracted cone scales into four classes: (1) First-year aborted ovules, (2) second-year aborted ovules, (3) empty seed, and (4) filled seed. After the losses in each class were estimated, specific causes of seed losses were sought.

First-year aborted ovules.--First-year aborted ovules are potential seeds that abort during the first growing season. In mature cones, therefore, they are no larger than normal ovules after one year of development. The wing develops normally, however, and these ovules are "wings without seeds."

First-year abortion of ovules was a major type of loss in slash pine and loblolly pine. In 1972 sample cones, slash pine lost 110 seeds per cone and loblolly pine lost 69 seeds as first-year aborted ovules. These losses represented 65 percent and 44 percent of the seed potential for these two species.

The high losses in this category made it very important to identify specific causes. Two causes were known: Pollination failure (Sarvas 1962, McWilliam 1959), and feeding by seedbugs, particularly Leptoglossus corculus (DeBarr and Ebel 1973). The early instars of this insect are able to penetrate the conelet, puncture the integument and extract large portions of the ovule. Feeding damage of this type can be identified macroscopically and confirmed by microscopic examination (Bramlett and Johnson 1975). When feeding of seedbug occurs for an extended period, the conelet may also abort (DeBarr and Kormanik 1975).

In addition to these established causes of first-year ovule abortion, other causes are suspected. Apparently, for unknown reasons, some pollinated ovules do not develop. Also, fungi may cause ovule deterioration (Miller and Bramlett 1975), but specific details are not yet established.

Second-year aborted ovules.--Second-year aborted ovules survive the first year of development but abort during the second year before the seedcoat is well formed. These ovules appear resinous, collapsed, or necrotic in early summer. Frequently, resin streaks are also visible in the vascular cells of the scale. In addition to the large necrotic ovules, some aborted ovules produce a small seed coat but are not as large as a fully developed seed.

Heavy losses of this type were observed on shortleaf and Virginia pines. Shortleaf pine averaged 41 second-year aborted ovules per cone (47 percent) and Virginia pine had 31 (36 percent) in samples from unprotected natural stands. Sample cones of loblolly and slash pine had 23 and 17 second-year aborted ovules per cone or 15 percent and 10 percent of the seed potential.

The major cause of second-year aborted ovules in pines is feeding by pine seedbugs on the developing cones. Krugman and Koerber (1969) described feeding damage of Leptoglossus occidentalis on ponderosa pine (P. ponderosa Laws.). DeBarr (1967) identified two seedbugs that damage southern pine seed. DeBarr and Ebel (1974) increased seed yield in shortleaf and loblolly pine with screen cages and described ovule damage associated with feeding of Leptoglossus corculus. Bramlett and Moyer (1973) were able to eliminate almost all second-year aborted ovules in Virginia pine with protective screen wire cages that excluded seedbugs prevalent in the area.

These observations show that seedbug damage is responsible for most second-year abortion of ovules. The cause of the smaller, hardened, aborted ovules is not known. Some preliminary indications associated this type damage with moisture or nutritional stress during development.

Empty seeds.--All seeds have some remnants of gametophyte tissue and/or a shriveled or damaged embryo. They were formerly classed as empty if they would float in 95 percent ethanol, and they are now usually recognized by X-ray detection. In a broad sense, seeds damaged by seedbugs or fungi, and seeds with abnormal development can be grouped as empty seeds.

In a well-managed seed orchard, 85 percent of the seeds should be filled. In the absence of protection from insects, values below 50 percent have been observed. Known causes of empty seeds include insects, fungi, and embryonic lethal alleles.

Insect attack and damage seed in the nearly mature or mature cone. Unlike cone-destroying insects such as Dioryctria spp., the leaf-footed pine seedbug and the shieldback seedbug feed on seeds without leaving external signs of damage on the cone. External damage on the seedcoat may also be minimal, even when the embryo is killed (DeBarr 1970).

Insects are not the only cause of empty seeds in pines. Even when protective screen cages are used to exclude seed insects, empty seeds are found in the sample cones (Bramlett and Moyer 1973; DeBarr and others 1975; DeBarr and Ebel 1973). Many studies have shown an increase in the percentage of empty seeds following self-pollination (Franklin 1970). These empty seeds are the result of recessive embryonic lethal alleles that cause embryo mortality soon after fertilization. Although pines may have polyzygotic embryony, selfing and other forms of inbreeding increase the probability of embryo abortion in the pine ovule. Empty seeds are the result (Bramlett and Popham 1971). Empty seeds also occur following cross pollinations or wind pollinations. Embryo mortality following cross pollinations (with screen cage protection) are apparently due to the random matching of the recessive embryonic lethals (Bramlett and Pepper 1974).

Other classes of seed loss or damage include seedworm (Laspeyresia spp.) damage, abnormality, fungal infection, and incompletely filled seeds. The cumulative total of these seed losses is usually less than 10 percent and normally is not a serious problem in the seed orchard.

Filled seeds.--Filled seeds have the potential to germinate; they have healthy, undamaged gametophyte tissue, a normal embryo, and no evidence of fungi or other destructive pests. They are the end product of the seed orchard and represents the final yield from an initial crop of female flowers. As previously stated, the ratio of filled seed to the seed potential gives the seed efficiency per cone. Seed orchard efficiency is calculated by combining seed efficiency and cone efficiency.

$$\text{Seed orchard efficiency} = \frac{\text{Cone}}{\text{Flowers}} \times \frac{\text{Filled seed}}{\text{Seed potential}} \times 100\%$$

$$= \text{Cone efficiency} \times \text{Seed efficiency} \times 100\%.$$

Seed orchard efficiency can be used to evaluate seed orchard performance. For example, an initial crop of 8,000 flowers per acre in a slash pine seed orchard would have a biological potential of 8,000 X 170 (seed potential) for slash pine or 1,360,000 seeds. Based on an average of 13,500 cleaned seeds per pound for slash pine, this yield would equal ca 100 pounds of seeds per acre. The fate of these seeds is determined by the amount of loss occurring during the development period. For example, under intensive management approximately 75 percent of the initial flowers could become mature cones. Losses would include not only insect damage, but other abiotic factors such as unharvested cones, wind damage, trees lost to lightning, etc. With a 75 percent cone efficiency and a similar 75 percent seed efficiency, the orchard would produce 56 percent of the biological potential, or 56 pounds of seeds per acre. This value may represent the upper limit that can be expected in seed orchards even under excellent management.

Under typical management, a seed orchard may produce 50 percent cone and seed efficiencies, but since seed orchard efficiency is the product of these two values, the overall efficiency is only 25 percent. Under poor or no management, seed efficiency may be below 10 percent. Cone efficiency X seed efficiency in a natural stand of shortleaf pine ranged from 0.5 to 17.4 percent during 5 years of observations and averaged about 4 percent (Bramlett 1972).

SEED EXTRACTION

The percentage of seed removed by extraction can be important to the overall seed efficiency. The primary factor is the degree of cone opening after heating in the extraction kiln. Poor cone opening may be associated with cones harvested too early, fungal damage, insect damage, or case hardening during storage. These causes combined

represent a net loss of seeds supplied to the nursery. The quantity of seeds lost during the extraction process can be estimated by comparing number of seeds extracted to the total number produced by the cone. This value is the extraction efficiency.

$$\text{Extraction efficiency} = \frac{\text{Number of seeds extracted}}{\text{Total number of seeds/cone}} \times 100\%.$$

SEED GERMINATION

When a seed germinates and produces a seedling, the final link between generations is complete. Germination is the subject of considerable literature but for this paper will simply be considered as the percentage of filled seeds that germinate normally during a specified test period. Germination efficiency (percent germinated) is determined for each cone.

$$\text{Germination efficiency} = \frac{\text{Number of germinated filled seeds}}{\text{Total number of filled seeds}} \times 100\%.$$

Germination of a pine seedlot is normally between 70 and 90 percent. The average germination efficiency value can be combined with previous efficiency values to estimate overall seed orchard to nursery efficiency.

SEED ORCHARD TO NURSERY EFFICIENCY

The ability of a seed orchard to produce not only seeds but seedlings can be evaluated as a product of the four separate efficiency statements, cone efficiency (CE), seed efficiency (SE), extraction efficiency (EE), and germination efficiency (GE). Thus, seed orchard-nursery efficiency (SO-NE) is:

$$\text{SO-NE} = \text{CE} \times \text{SE} \times \text{EE} \times \text{GE}.$$

Using this combined efficiency value, the seed orchard manager can evaluate the overall performance of a given flower crop to produce seedlings for the nursery. With excellent seed orchard management and protection, efficiency values of 75 percent are possible for CE and SE and 90 percent for EE and GE. Thus, SO-NE = 0.75 x 0.75 x 0.90 x 0.90 = 0.45. This value of 45 percent efficiency may approach the upper biological limit for an operational seed orchard. Levels of SO-NE below 45 percent indicate that some improvements may be possible.

Godbee and others (1977) used SO-NE to evaluate an insecticide spray program in a Georgia Forestry Commission seed orchard. The SO-NE averaged 17 percent on plots treated with the insecticide, compared to 6 percent in the control plots. This evaluation indicated that the spray program was increasing the seeds and seedlings produced

in the orchard, yet substantial losses were still occurring. Modifications in the insect control program have now been implemented to provide more effective control.

Evaluation of seed orchard performance by computing efficiency values suggests two general ways to increase the seed production of seed orchards. The first is to increase flower production while holding values for CE, SE, EE, and GE constant. The second is to reduce seed losses during one or more of the developmental stages. The logical approach to reduce seed losses is to first evaluate seed production efficiency and identify and quantify the specific causes of seed losses. Once the seed losses are known, control measures can be prescribed to prevent or reduce the losses. If the orchard manager knows the impact of a specific loss and the cost of the control, he can evaluate the projected increase in seed efficiency and seed yield.

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FLOWER PRODUCTION ON CLONAL ORCHARDS AT OCONTO RIVER SEED ORCHARD IN WISCONSIN

J. G. Murphy and R. G. Miller[1]

ABSTRACT.--The Eastern Region, USDA Forest Service has been establishing and managing seed orchards to produce improved seed for the National Forests in the Lake States since 1969. This paper presents a review of the female flower production for the past 4 years in the white pine, white spruce, and black spruce clonal seed orchards.

The tree improvement program being conducted on the National Forests in the Lake States has been described in detail (Miller and Murphy 1976). The Lake States National Forests have been divided into nine seed collection zones and four breeding zones (fig. 1). Beginning in 1969, seed orchards were established by breeding zone for selected species at the Oconto River Seed Orchard complex located near Langlade, Wisconsin. The number of ramets required to produce anticipated seed needs in the various orchards were based on research information (Nienstaedt and Jeffers 1970) and best estimates of potential production. Information on cone production, frequency of crops, and flowering of individual clones is needed to make better estimates of potential cone production in large scale seed orchards for white spruce, black spruce, and white pine. Female flower data collected between 1973 and 1977 will be reported for two white pine, four white spruce, and two black spruce clonal seed orchards.

SEED ORCHARDS

Following is a brief description of the plant materials included in the seed orchards (table 1).

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Table 1.--Individual seed orchard statistics

Species	Orchard no.	Breeding zone[1/]	Clones	Grafts	Acres	Year of establishment	Year of first flowering
			- - - Number - - -				
White pine	1	All	798	3,612	40	1969	1973
White pine	3	All	36	830	10	1969	1973
White spruce	4	A	56	903	21	1969	1974
White spruce	6	B	50	1,128	21	1969	1974
White spruce	14	C	40	1,128	14	1973	1974
White spruce	16	All	39	220	5	1973	1974
Black spruce	8	A	50	1,501	21	1971	1974
Black spruce	10	B	47	778	20	1973	1974

1/ Breeding zones are shown in figure 1.

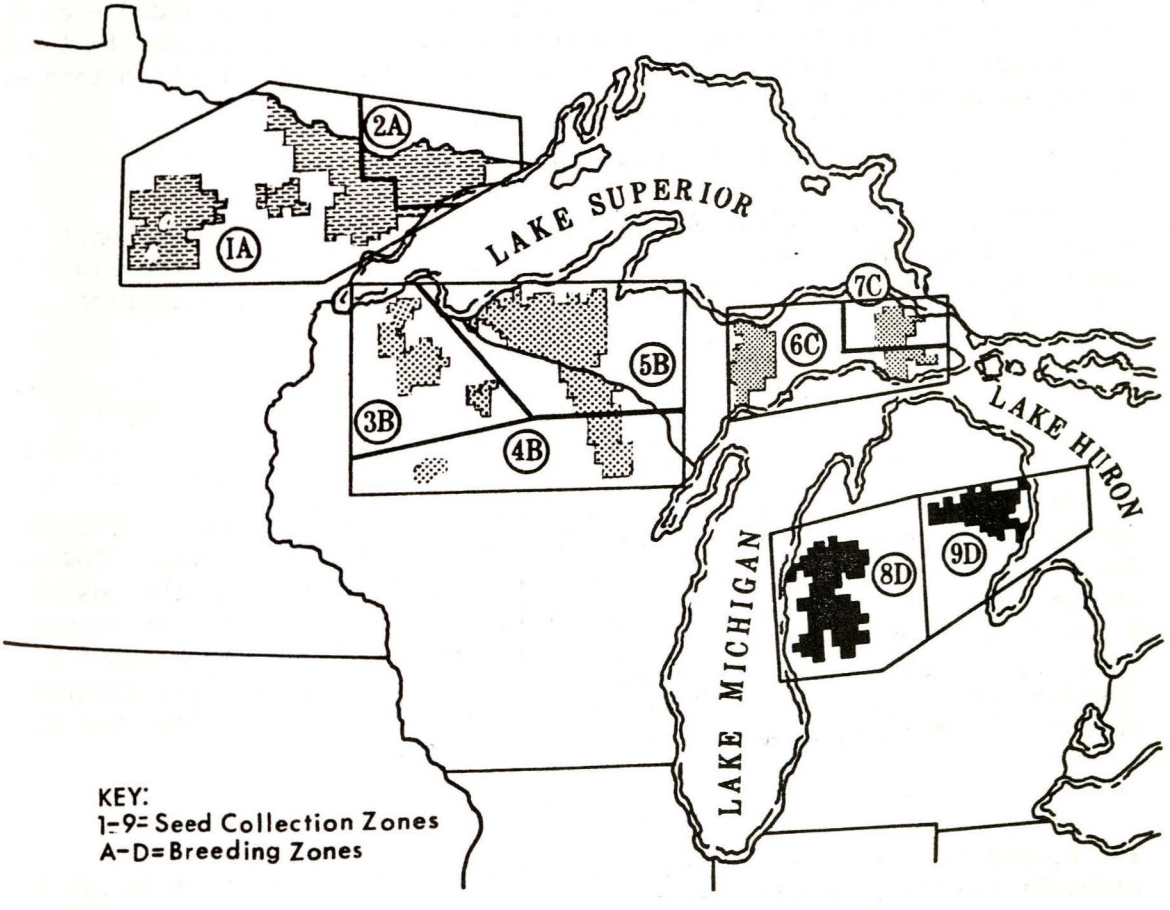

Figure 1.--Seed collection zones and breeding zones.

White Pine Orchards 1 and 3

Orchard 1 (white pine breeding arboretum) contains ramets from 798 ortets selected for their apparent resistance to white pine blister rust. The majority of the ortets came from the Lake States; however, a few came from Eastern States.

Orchard 3, which is an interim seed orchard, contains five clones selected by Drs. Riker and Patton, University of Wisconsin, and 31 clones selected by Drs. Heimburger and Zsuffa, Maple, Ontario, Canada. The majority of orchard 3 was established in 1969 whereas orchard 1 was established over a 5 year period.

White Spruce Orchards 4, 6, 14, and 16

The plants in orchards 4, 6, and 14 were selected on National Forests in breeding zones A, B, and C, respectively. The material in orchard 16 was selected by personnel of the North Central Forest Experiment Station in Rhinelander, Wisconsin, from ortets originating from seed collected in the Ottawa River Valley in Ontario, Canada, and growing in provenance tests. All of the white spruce orchards have had ramets added each year since the original planting. Orchard 4 suffered large losses following the second year planting, resulting in much more age variation than the other white spruce orchards.

Black Spruce Orchards 8 and 10

Plant materials in orchards 8 and 10 were selected on National Forests in breeding zones A and B, respectively. Both of the black spruce orchards have had ramets added each year since the original planting.

DATA

Collection

The flowering data for white pine and white spruce were collected in the spring as part of the breeding program. The black spruce flowering data were determined by cone collections in the fall with the possibility of some flowers being lost due to frost. All flower data are recorded as 1: none, 2: female, 3: male, and 4:male and female for each ramet and then summarized using the Region 9 tree improvement computer programs. The data do not account for the quantity of flowers produced, but only whether a ramet has produced one or more flowers.

Computation

The number of living trees and number of clones represented in all the orchards varies from year to year because the orchards were established

over a period of time. Therefore, the data were converted to percent of trees and percent of clones that produced female flowers. The data represent the following years: white pine, 1973-1976 (table 2); white spruce, 1974-1977 (table 3); and black spruce, 1974-1976 (table 4). The frequency of clones producing flowers during the data collection period was calculated for orchards 3, 4, and 6 (table 5).

Table 2.--Living ramets and clones with female flowers for white pine

(In percent)

Orchard no.	Type of material	Year			
		1973	1974	1975	1976
1	Ramets	19	18	4	4
	Clones	36	34	10	11
3	Ramets	21	31	18	6
	Clones	69	83	69	39

Table 3.--Living ramets and clones with female flowers for white spruce

(In percent)

Orchard no.	Type of material	Year			
		1974	1975	1976	1977
4	Ramets	24	17	12	33
	Clones	79	46	39	92
6	Ramets	20	19	9	75
	Clones	85	82	60	100
14	Ramets	71	20	16	55
	Clones	91	89	80	98
16	Ramets	34	8	5	44
	Clones	64	41	21	66

Table 4.--Living ramets and clones with female flowers for black spruce

(In percent)

Orchard no.	Type of material	Year 1974	1975	1976
8	Ramets	(No data)	28	29
	Clones	(No data)	94	90
10	Ramets	41	33	21
	Clones	92	74	89

Table 5.--The frequency of clones producing flowers over a 4-year period

(In percent)

Seed orchard and species	Number of years out of four 4	3	2	1	0
3 White pine	30	39	6	11	14
4 White spruce	29	23	34	9	5
6 White spruce	46	42	8	4	0

Correlation coefficients were computed between percent of living trees with flowers and ortet age for orchards 4 and 6 (table 6).

Table 6.--Correlation coefficient of percent of living trees with flowers and ortet age1/

Orchard no.	Year 1974	1975	1976
4	+.04	-.22	-.25
6	+.24	+.16	+.28

1/ Not significant at the 95 percent level.

For the data available for each orchard, correlations were computed between a month's total rainfall by year and the following year's percent of ramets producing flowers. This was done for the months May through September (table 7).

Table 7.--Correlation coefficient of percent of living ramets with flowers and the previous years rainfall by months

Species and flower data year	Orchard no.	Month				
		May	June	July	Aug.	Sept.
White pine (1973-1976)	1	+.23	-.96[1/]	+.64	+.28	-.08
	3	+.67	-.73	+.40	-.09	-.70
White spruce (1974-1977)	4	+.24	-.81	-.56	-.82	-.86
	6	-.19	-.48	-.78	-.72	-.73
	14	+.77	-.97[1/]	-.17	-.80	-.62
	16	+.41	-.88	-.53	-.91	-.76
Black spruce (1974-1976)	10	+.91	-.95[1/]	+.78	-.40	-.91

[1/] Significant at the 95 percent level.

DISCUSSION

The flowering trend for the eight seed orchards between 1973/74 and 1976 shows a decline in the number of ramets and number of clones that produced flowers. The decline may be due to normal cyclic production. A review of the amount of seed produced from the flowering years indicated similar results. In all four of the white spruce orchards a total of 3.00 lbs. of seed was produced in 1974, 0.25 pounds in 1975, and 0.002 pounds in 1976. However, 1977 was the best flowering year to date in the white spruce orchards. Data on white pine and black spruce for 1977 flowering have not been tabulated, but observations indicate that flowering is good in these orchards as well.

A review of the computer data summaries for each of the orchards revealed a large amount of variation among clones in year-to-year production. Many clones produce consistently but a few clones never have and maybe never will produce flowers. Correlation coefficients for age of ortet (22 to 135 years) and flower production ranged from +0.28 to -0.25 (table 6), indicating that for this age group there was no relation. However, this data does not reflect the ages (3 to 6 years) of the ramets within orchards.

It is known that moisture stress can be an important factor in flowering and cone production (Sweet 1975). Correlation coefficients were computed to determine if there was a relation between total rainfall for a given month and the next year's flower production. During the summer of 1976, the seed orchard was subject to a prolonged drought. Weather records for 1976 show that the May through September rainfall was 46 percent below the average of the previous 4 years. We hoped that the drought would stimulate flower production in the seed orchards for 1977; the white spruce data show that flowering was dramatically increased in 1977 (table 3). The results for the seven orchards indicate that a correlation between June rainfall and flower production may exist (table 7). However, one must consider that this data only includes a single severe drought year and the results may be due to normal cyclic flower production.

CONCLUSIONS

Although the data presented here only covers a few years of production, it is encouraging to note the number of clones that have produced cones. Within a few years these orchards should produce sufficient seed to meet the needs of the National Forests in the Lake States.

The possible correlation between flowering and rainfall must be considered with other factors when selecting seed orchard sites, and will be useful in planning breeding work and cone collection operations. This relation could also become the basis for determining when to apply a flower induction treatment such as fertilizer. This may be a key to producing large amounts of flowers for breeding and production needs on a more regular basis.

In the future we will continue to observe the flowering-moisture relation. We may find that in order to be useful, correlations will have to be established for each individual orchard and perhaps even for individual clones.

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SEED PRODUCTION IN THE FIRST EIGHT YEARS AND FREQUENCY OF NATURAL SELFING IN A SIMULATED JACK PINE SEEDLING SEED ORCHARD

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ABSTRACT.-- Seed production and percent of natural selfing were determined in an 8 x 8-foot, 1200-tree plantation. Actual seed production was determined through age 6; production through age 8 was projected based on first-year cone counts at age 7. The percent of natural self-pollination, production of seedlings from natural selfing, and percent of selfs that were lethal were determined by marker seedlings. Ovulate strobili production began at 17 months of age; by age 4 a few trees produced small quantities of mature cones and seed. By age 8 years most trees produced an average of 39 mature cones per tree, 26,200 cones per acre, or 655,000 filled seed per acre. Total filled seed per acre produced through age 8 years was 1,159,000. The overall average of open-pollinated seedlings resulting from natural selfing was about 7 percent. The frequency of natural self-pollination, determined from carriers of selected seedling marker types, ranged up to 34 percent; on the average more than half of these were lethal. Removing trees showing high selfing percentages and careful culling of poorer seedlings grown from orchard seed before field planting should reduce the impact of natural selfing on wood yield in such plantations to a minimum.

Three important considerations for managing pine seed orchards are: how early do they produce seed, how much seed do they produce, and how much seed results from selfing?

Jack pine (Pinus banksiana Lamb.) begins to produce ovulate strobili in the third and fourth year when grown under standard nursery conditions (Righter 1939, Wright 1964, Rudolph 1966b), but as early as 17 months if started in the greenhouse under near-optimum conditions (Rudolph 1966b,

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Jeffers and Nienstaedt 1972). Once cone production begins it is fairly regular (Godman and Mattson 1976) and increases until crown competition becomes a factor (Roe 1963).

Jeffers (1976), using well-substantiated seed yields per cone (Schantz-Hansen 1941, Rudolf 1958, Roe 1963, Rudolph 1966b, 1967, Jeffers 1972), and actual female strobili per tree at age 5, predicted a yield of 267,000 full seed per hectare of jack pine seed orchard at age 6.

Most wind-pollinated seed produced on jack pine results from cross-fertilization but up to 25 percent naturally selfed seed has been reported in progenies of individual trees (Fowler 1965a, 1965b, Rudolph 1966a, Teich 1970, Sittmann and Tyson 1971). Hadders and Koski (1975) have reviewed the factors affecting the proportion of natural selfing in relation to production of seed in pine seed orchards. Selfing can result in reduced cone set, lower filled seed yield, poorer seed germination, lower survival of seedlings, depressed growth rate, and increased frequency of deviant types (Fowler 1965b, Rudolph 1966a, 1967, 1976), all undesirable features in seed orchard management.

In this paper I report the actual seed production in a simulated seedling seed orchard through age 6 from seed, the projected yields through age 8 based on first-year cone counts at age 7, and I estimate the frequency of natural selfing based on the proportion of various marker seedling types in open- and controlled self-pollinated progenies. I also recommend the means to reduce the effect of natural selfing on future wood yields.

MATERIAL AND METHODS

Trees for this study were grown from seed collected in August, 1960 from approximately 50 jack pine trees in an extensive stand near Rhinelander, Wisconsin. Variable quantities of seed per tree comprised the bulk lot. The primary objective of the study was to determine the effects of gamma irradiation of the seed at various exposure levels on the long-term survival and reproductive capacity. However, the design of the study, particularly of the field test, lends itself well for consideration as a simulated seed orchard. The radiation effects, which will be reported elsewhere, do not invalidate the seed production results to be presented here but do make the estimates slightly more conservative than could be expected without irradiation due to slightly lower production in the highly irradiated lots. The slight impact of irradiation noted on seed yield and self-ability will be discussed in the results section. Design details relating to radiation treatments will only be presented to put the total experiment into perspective.

In December, 1966, thirteen samples of 300 seeds each were fully imbibed in water. They were then gamma irradiated at one of thirteen exposures ranging to 19,200 R. The irradiated seedlots were divided into three replications of 100 seeds each and sown in sand in a greenhouse. At 1 month of age, 125 seedlings per exposure randomly chosen from the combined three replications were transplanted into 2½ in. x 2½ in. x 3 in. veneer plant bands, and randomly assigned to five replications of 25 seedlings each. The seedlings were grown in the greenhouse until May 1967 when they were transplanted to the Hugo Sauer Nursery near Rhinelander, Wisconsin. The same design as in the greenhouse was followed in the nursery but with a 7 in. x 7 in. spacing between plants. Incidence of flowering was scored on the nursery-grown seedlings in the spring, 1968 and percent of trees flowering and ovulate conelets per tree determined.

Approximately 1200 seedlings survived and were field planted near Lake Tomahawk, Wisconsin on June 10, 1969 at an 8 ft. x 8 ft. spacing inthe same randomized block design as was used in the nursery with 5 x 5 tree plots.

All open-pollinated cones produced in 1970, 1971, and 1972 (ages 4, 5, and 6 years) were collected separately from each tree each year. Seed was extracted by shaking the cones in a sieve with 1/4 in. x 1/4 in. openings following a brief dip in boiling water to open the cone scales. The seed was counted and X-rayed to determine percent filled. Percent of trees with mature cones and seed yields were determined.

In the spring, 1973, when the trees were in their seventh growing season, the total number of ovulate conelets per tree was scored on the center nine trees of each plot to facilitate estimation of cone production in 1974 or at age eight. Also in the spring, 1973, two trees that had relatively abundant male and female strobili production were selected in each plot for controlled self-pollinations. Whenever possible, trees in the same position within plots were selected to provide for a relatively uniform but representative selection of trees throughout the planting. Thus, a total of ten trees per each of the twelve radiation treatment levels (no trees survived in the 19,200 R treatment) or 120 trees were selected for the self-pollinations. Five isolation bags were placed on each tree and the female strobili in each of the 600 bags were self-pollinated on June 1 and 2, 1973. Mature cones from the self-pollinations were collected in late September, 1974, keeping those from each pollination bag separate. Five open-pollinated cones from each of the bagged trees were collected at the same time. Of the 120 trees self-pollinated, 106 produced a total of 1200 cones.

The selfed seed together with the sample of open-pollinated 1973 seed from five cones on the same trees was extracted by hand by removing the cone scales, counted, X-rayed to determine percent filled, and germinated in sand in the greenhouse. The seedlings were examined at frequent intervals for 3 months to search for characteristics with potential as genetic markers. Frequency of markers within individual tree progenies was expressed as a percent of total seedlings germinated.

A similar procedure for screening for marker seedlings was used to screen open-pollinated seed from all trees producing cones in 1972. Whenever possible, 200 filled seed from each of the 1178 trees producing cones was sown; however, fewer filled seeds were available from some trees. A "damping-off" fungus problem in one greenhouse bench during germination reduced the number of open-pollinated families that could be effectively screened to 848.

Percent of open-pollinated seedlings resulting from natural self-fertilization was determined for all families that were segregating for the various types of marker seedlings. It was assumed for simplicity, that the markers were recessive and that they segregated in a 3:1 ratio upon selfing; that is, the frequency of marker seedlings in the open-pollinated progenies was multiplied by four. The simplistic 3:1 ratio was assumed in this study because a genetic explanation for apparent deviations from this ratio observed in some families is not known.

Percent of natural self-pollination was calculated as the ratio:

$$\frac{\text{percent of marker seedlings in open-pollinated progenies}}{\text{percent of marker seedlings in controlled self-pollinated progenies}}$$

Application of this ratio assumes that self-pollen is as viable and active as cross-pollen and that one embryo occurs per ovule. It must be recognized that the percent of natural self-pollination determined in this manner may be over-estimated to an unknown degree due to possible mating between carriers of common markers.

The percent of self-pollinations that were lethal was determined from the relationship:

$$\frac{\text{(percent of natural self-pollination)} \text{ minus } \text{(percent of open-pollinated seedlings from natural self-fertilization)}}{\text{percent of natural self-pollination}}$$

RESULTS AND DISCUSSION

Cone and Seed Production

Ovulate strobili production began in the spring, 1968, in the nursery at approximately 17 months of age, on 28 percent of the trees (table 1). Of the total surviving trees, 21 percent had one, 6 percent had two, and less than 1 percent had three or four ovulate strobili per tree. Percent of trees flowering was significantly lower in some of the highest radiation levels. Thus, the overall average percent of trees flowering was below what might have occurred without irradiation (table 1). However, even with this decrease, the percent of trees producing ovulate strobili in this population is somewhat higher than that previously reported for 17-month-old seedlings (Rudolph 1966b, Jeffers and Nienstaedt 1972). Less than one percent of the trees produced male strobili at this age.

Table 1.--Ovulate strobili production in the nursery in 1968 on 17-month-old jack pine seedlings field planted into a simulated seed orchard the following year

Gamma exposure (R)	Total trees	Trees with: One ovulate strobilus	Two ovulate strobili	Three ovulate strobili	Four ovulate strobili	Total trees flowering:	
	Number	Percent				Number	Percent
0	112	28.6	1.8	2.7		37	33.0
150	117	20.5	12.0	1.7		40	34.2
300	102	23.5	8.8			33	32.4
600	120	25.8	2.5			36	30.0
1200	114	13.2	4.4			20	17.5
1800	96	22.9	4.2	1.0		27	28.1
2400	108	26.8	8.3	1.8		40	37.0
3600	100	15.0	4.0		1.0	20	20.0
4800	107	15.9	4.7			22	20.6
7200	90	23.3	4.4	1.1		26	28.9
9600	84	17.8	2.4	1.2		18	21.4
14400	49	16.3	8.2			12	24.5
Total or Mean	1199	20.8	5.5	0.8	0.1	331	27.6[1/]

1/ Based on total trees flowering divided by total number of trees.

Practically all the cones aborted and no cones were collected in 1969. This was probably due to the transplanting to the field in June, 1969, and to the lack of pollen in the nursery in 1968. Probably for the same reasons, the percent of trees with mature cones at age four years in 1970, less than 16 percent, was only slightly over one-half as many as produced ovulate strobili in 1968 and less than two mature cones per flowering tree were produced (table 2). The ovulate strobili primordia for the 1970 cones were differentiated during the 1968 growing season in the nursery and possibly suffered high abortion from transplanting shock and lack of pollen in 1969. Similarly, only 10 seeds were produced per cone and only 23 percent of these were filled.

In 1971, at age 5, the proportion of trees producing cones increased to about one-half but the number of cones per cone-bearing tree was still only about two (table 2). However, the number of filled seeds per cone, seven, was three times that in 1970 but still far below normal even though more than two-thirds of the total seed was filled. This possibly resulted from a meager supply of pollen in 1970. The number of filled seeds produced per acre in 1971 increased to more than 12 times that in 1970.

A more abundant pollen supply on the orchard trees in 1971 was apparent because in 1972 at age 6, 22 seeds per cone were produced and three-fourths of them were filled (table 2). This, coupled with an increase in percent of trees producing cones to 93, and more than ten cones per tree, increased the yield of filled seeds per acre to more than 113,000. The total yield of filled seed per acre through age six years was more than 119,000.

Actual production per acre was higher than predicted by Jeffers (1976), who used ovulate strobili at age 5 for his prediction of production at age 6, even though there were only 681 trees per acre compared to 3000 in Jeffers' study. This was largely due to more cones per tree than predicted which was only partially offset by a lower yield of filled seed per cone, 16.5, rather than the 25 predicted by Jeffers (1976). As noted earlier, the relatively few filled seeds per cone at age 6 is due at least partially to insufficient pollen. Mass artificial pollination with pollen from selected trees would likely increase substantially the yield of filled seed in such young orchards. By age 7, there was enough pollen to adequately pollinate the ovulate cones that matured at age 8.

Yields of filled seed during the first 6 years in this study were also lowered by the choice of transplanting time in June, 1969. Transplantation to the field at this time possibly not only decreased the percent of trees with cones in 1970 but likely had an impact on the production in the next 2 years as well (table 2). If the seedlings had been transplanted in the late summer of 1968 when the primordia

Table 2.--Mature cone and seed production of jack pine trees 4, 5, and 6 years old from seed

Year	Total trees	Trees w/mature cones	Cones per tree: All trees	Cones per tree: Trees with cones	Seed per tree: All trees	Seed per tree: Trees with cones	Filled seed per tree: All trees	Filled seed per tree: Trees with cones	Seeds per cone	Filled seeds per cone	Total seed filled	Filled seeds per acre at 8 ft x 8 ft spacing
	Number	Percent	Number								Percent	Number
1970	1187	15.6	0.3	1.7	2.8	17.7	0.6	4.1	10.4	2.4	23.3	439
1971	1183	47.0	1.1	2.4	11.6	24.7	8.0	17.0	10.2	7.0	68.6	5,429
1972	1178	93.0	10.1	10.9	221.2	237.8	166.7	179.2	21.9	16.5	75.4	113,513
TOTAL FILLED SEED PER ACRE PRODUCED IN THE FIRST 6 YEARS												119,381

for the 1969 female strobili were already differentiated--but in time to establish the seedlings in the field before winter dormancy--there would have been more 1969 female strobili and more differentiation of 1970 and 1971 strobili. In addition, pollen production could possibly have been greater, resulting in higher seed yields. However, further research is needed to verify the impact of transplanting time on early seed production.

The rapid subsequent rise in cone and seed production beyond age 6 years when the trees were better established in the field and pollen production was more abundant is evident from conelet counts made in 1973 at age 7. Almost 43 conelets per tree were found after natural pollination in the summer, 1973 after early abortion and/or abscission had occurred. Assuming an additional 10 percent cone loss between the first year and cone maturity (Rudolph 1976), about 39 cones per tree could be expected in 1974 at age 8. Using this information and the actual seed yields for the first 6 years the total yields of filled seeds through age 8 may be summarized as follows:

Total filled seed production per acre in 1970 (age 4), 1971 (age 5), and 1972 (age 6), (from table 2)	119,381
Ave. number of conelets per tree in summer, 1973 (age 7)	42.75
Number of cones per tree maturing in 1974 (age 8) assuming additional 10 percent cone loss	38.48
Number of cones per acre in 1974 (age 8) at 8 ft x 8 ft spacing (681 trees)	26,205
Number of filled seeds per tree in 1974 (age 8) assuming 25 filled seeds per cone	962
Number of filled seed per acre in 1974 (age 8) at 8 ft x 8 ft spacing (681 trees)	655,122
Number of filled seed per acre in 1973 (age 7) assuming mid-point between 1972 and 1974	384,317
Total filled seed production per acre through age 8	1,158,820

As pointed out earlier, the yields given above are somewhat lower than may ordinarily be expected with the same cultural methods due to the decreased yields at the higher radiation levels. Nevertheless, they indicate that substantial seed yields in jack pine seedling seed orchards can be expected 8 years from seed. Assuming that at least 90 percent of the filled seed is viable (personal observation), about

1 million seedlings per acre of seed orchard can be produced within 8 years. Higher yields could be expected with improved cultural methods and attention to avoiding transplanting shocks. Selection of trees for serotinous cones would permit storing earlier seed production on the trees until yields are high enough to make collections more economically feasible, perhaps beginning at age 8. Selections and thinning could then be applied to promote maximum cone and seed production on the remaining trees in subsequent years.

Frequency of Natural Selfing

Estimated percent of open-pollinated seedlings from natural self-fertilization averaged about 7 percent assuming the markers were a recessive trait segregating 3:1 upon selfing (table 3). This is based on the evaluation of 24 different markers within 567 open-pollinated families among 848 families screened. The range was from about 2 to 18 percent. Proportion of natural self-pollinations, not all of which would result in successful self-fertilization and selfed seedlings, cannot be estimated from open-pollinated progenies.

Note that the proposed markers from open-pollinated progenies were scored on seedlings 3 months or less old. Therefore, some of the supposed markers, particularly certain of the dwarf types such as the "typical" green dwarf (table 3), need to be verified when the seedlings are considerably older. This classification included all seedlings that showed greatly reduced growth at this young age; some of these slow-growers probably exhibited temporary non-genetic effects such as differences in seed size. Additional development will need to be observed and will undoubtedly result in a reduced number of carrier trees for dwarfism. Other characteristics now classified as markers may similarly prove to be temporary physiological effects with age.

Our previous experience has shown that two marker types, yellow-green cotyledons and light green cotyledons, are under rigid genetic control. And since both open- and controlled self-pollinated progenies were available with these markers, they were chosen for more detailed analysis of natural self-pollination frequency in open-pollination and lethality following self pollination (tables 4 and 5).

The average percentages of marker seedlings in open-pollinated progenies of trees carrying these two types of markers and used in the detailed analysis--3 and 5+ percent for the yellow-green and light green cotyledon types, respectively--were slightly higher than the average for all trees carrying these markers (compare table 3 with tables 4 and 5). An average of about 12 and 8 percent of the open-pollinated seedlings in progenies with the two marker types resulted from natural selfing (tables 4 and 5). The lower than expected estimates (assuming 3:1 segregation) of the percent of open-pollinated seedlings resulting from natural selfing in the light green cotyledon

Table 3.--Estimation of proportion of open-pollinated seedlings resulting from natural selfing from frequency of marker seedlings in 1972 open-pollinated progenies

Marker type	Carrier trees 1/	Marker seedlings within family	Open-pollinated seedlings from natural selfing 2/
	Number	Average percent	Percent
White cotyledons	4	2.0	8.0
Yellow-green cotyledons	28	2.6	10.4
Greenish-yellow cotyledons	9	1.2	4.8
Whitish-green cotyledons	6	4.2	16.8
Greenish-white cotyledons	4	1.7	6.8
Light green cotyledons	13	1.2	4.8
Yellow-green primary leaves	4	2.6	10.4
Bluish-green dwarf (typical)	75	1.3	5.2
Green dwarf (typical)	258	2.0	8.0
Bluish-green dwarf(extremely small)	5	1.1	4.4
Green dwarf (extremely small)	9	0.8	3.2
Short, fine primary leaves	1	1.2	4.8
Bluish-green dwarf w/early bud set	21	1.7	6.8
Thin, fine, short primary leaves	3	1.1	4.4
Bluish-green dwarf with thick, stiff primary leaves	7	1.3	5.2
Stem forked immediately	2	4.5	18.0
No apical meristem	8	1.8	7.2
Cotyledons emerge through seedcoat	72	2.2	8.8
Double embryo	18	1.5	6.0
Tightly fused cotyledons	2	3.0	12.0
Kinky stem	6	0.6	2.4
Reverse germination	9	1.8	7.2
Possible chimera (two shoot types)	2	0.7	2.8
Twisted and deformed primary leaves	1	0.6	2.4
Total	567 3/		
Mean		1.78	7.12

1/ Based on 848 parent trees screened.
2/ Assuming marker is a recessive trait segregating 3:1 upon selfing.
3/ 132 trees are carriers of more than one marker type.

marker type are at least partially due to three factors: (1) unexplainable large deviations from a 3:1 segregation ratio such as in tree 4-150-14 (table 5); (2) similar or higher percentages of marker seedlings in open- and self-pollinated progenies of some trees such as tree 4-7200-18 (table 5); and (3) possible differences in linkages of the marker in different trees with different lethal factors. Sorenson (1969, 1971) has found such distorted ratios due to embryonic lethals.

Estimates of the average proportion of natural self-pollination are over 34 percent as determined from the yellow-green cotyledon marker type (table 4) and over 20 percent as determined from the light green cotyledon type (table 5). However, in both cases, if a 3:1 segregation ratio for the markers is assumed, the average percent of lethals resulting from self-pollination is more than 50. This results in an average production of open-pollinated seedlings from successful self-fertilizations of from 8 to 12 percent and is sligthly higher than natural selfing in slash pine reported by Kraus (1975). The probability is that only one-fourth of these are homozygous recessive for any given deleterious gene. Thus, only 2 to 3 percent of the open-pollinated seedlings in a seed orchard as described here should be deleteriously affected by self-pollination and successful self-fertilization, even though the percentages of seedlings from selfing would be 4 times more. The percentages of natural selfing, as estimated in this study by the use of marker seedlings, may be slightly inflated in that marker seedlings resulting from possible crosses between carriers of common alleles are indistinguishable from selfed seedlings. The occurrence of trees with common marker alleles cannot be verified without controlled cross-breeding.

Such low percentages of seedlings showing the depressing effects of self-pollination may not be significant in production of genetically improved seedlings from seed orchards although occasional trees showing high selfing percentages should be removed from the orchard. Culling of this small proportion of poorer seedlings from the progenies before field planting should be easily possible. The small numbers of weak seedlings that remain would likely succumb soon under the more rigorous environmental conditions in present-day tree plantations and would have little influence on ultimate wood yield from such plantations.

ACKNOWLEDGMENT

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Table 4.--Estimation of proportion of seedlings resulting from natural self-fertilization, frequency of natural self-pollination, and percent of selfs that are lethal from frequency of seedlings with yellow-green cotyledons in open- and controlled self-pollinated progenies

Tree no.	Seedlings that are: Open-pollinated	Seedlings that are: Self-pollinated	Marker seedlings: Open-pollinated	Marker seedlings: Self-pollinated	Open-pollinated seedlings from natural selfing 1/	Natural self-pollination 1/	Selfs that are lethal 1/
	Number		Percent				
1-600-3	229	112	2.6	6.2	10.4	41.9	75.2
2-1200-13	41	156	7.3	24.4	29.2	29.9	2.4
2-2400-13	125	38	2.4	2.6	9.6	92.3	89.6
2-3600-3	237	270	3.4	16.7	13.6	20.4	33.2
3-150-8	250	86	2.4	8.1	9.6	29.6	67.6
3-300-13	159	86	1.9	2.3	7.6	82.6	90.8
3-7200-8	166	76	3.6	15.8	14.4	22.8	36.8
3-7200-13	181	28	2.8	10.7	11.2	26.2	57.2
4-1200-3	197	109	1.0	4.6	4.0	21.7	81.6
4-4800-3	323	75	3.4	20.0	13.6	17.0	20.0
4-4800-18	145	238	2.8	7.1	11.2	39.4	71.6
4-9600-3	195	102	0.5	42.2	2.0	1.2	-- 2/
5-150-3	241	13	3.3	7.7	13.2	42.9	69.2
5-1200-18	172	268	3.5	14.6	14.0	24.0	41.6
5-1800-18	82	62	3.7	14.5	14.8	25.5	42.0
Total	2,743	1,719					
Mean			2.97	13.17	11.89	34.49	55.63

1/ Assuming marker is a recessive trait segregating 3:1 upon selfing.

Table 5.--Estimation of proportion of seedlings resulting from natural sel -fertilization, frequency of natural self-pollination, and percent of selfs that are lethal from frequency of seedlings with light green cotyledons in open- and controlled self-pollinated progenies

Tree no.	Seedlings that are: Open-pollinated	Seedlings that are: Self-pollinated	Marker seedlings: Open-pollinated	Marker seedlings: Self-pollinated	Open-pollinated seedlings from natural selfing 1/	Natural self-pollination 1/	Selfs that are lethal 1/
	Number		Percent				
1-1200-3	118	127	3.4	22.8	13.6	14.9	8.8
1-2400-3	213	87	1.9	9.2	7.6	20.7	63.2
3-150-18	239	158	0.8	5.1	3.2	15.7	79.6
3-300-13	159	86	2.5	8.1	10.0	30.9	67.6
4-150-14	214	192	1.9	55.4	7.6	3.4 2/	-- 3/
4-7200-18	153	43	21.6	14.0	-- 3/	-- 3/	44.0
Total	1,096	693					
Mean			5.35	19.10	8.40	20.55	52.64

1/ Assuming marker is a recessive trait segregating 3:1 upon selfing.

2/ Not included in mean due to large deviation from 3:1 ratio.

3/ Cannot be determined on basis of 3:1 ratio.

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ROOTING OF CONIFER PROPAGULES

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ABSTRACT.--An outline of the general problems involved with the propagation of elite conifer clones by rooted cuttings is drawn from published reports. New approaches for resolving these problems can come from studies of clone production through tissue culture methods. Probable extension of tissue culture techniques will permit the establishment of clones from adult, proven trees and may also provide a tool for *in vitro* propagule evaluation of disease resistance.

Reforestation by vegetative propagation of select clones offers the possibility of rapid improvement of forest yield (Shelbourne and Thulin 1974, Kleinschmit 1974). Clonal reforestation will require a fresh consideration of forest management and the implied biological risks, but the potential for utilization of non-additive genetic variance in trees and the benefit where seed production is slow or inadequate are both strong motives for operational vegetative propagation. Two methods of vegetative propagation offer promise: (1) rooted cuttings or needle fascicles, and (2) rooted propagules produced through tissue culture. The potentials and problems for these two approaches are compared in this paper.

Rooted Cuttings

A comprehensive review of the various reforestation programs using clonal rooted cuttings is presented by Brix and van den Driessche (1977). Clone production by rooted needle fascicles of pine was reviewed by Girouard (1971). General review of rooting practice for horticulturally important species is treated in the book by Hartmann and Kester (1975). Clonal propagation of *Cryptomeria japonica* has been used for centuries in the forests in Japan and provides the only data concerning long-term benefits and problems. Major programs for propagation of Norway spruce with rooted cuttings for operational planting are underway in West Germany (Kleinschmit 1974) and Finland

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(Lepistö 1974). A major research effort with radiata pine is continuing in New Zealand and Australia (Sweet and Wells 1974, Shelbourne and Thulin 1974) but operational plantings are not yet a reality. Research programs for Douglas-fir (Ross 1975), loblolly pine (van Buijtenen et al. 1975) and western hemlock (Boyd 1976) are being conducted in the United States and for white spruce and black spruce in Canada (Rauter 1974).

The above programs are new in terms of forest tree harvest cycles and most of the information deals with rooting behavior and the early field performance of the propagules. Long-term field behavior will be forthcoming. Production of rooted cuttings is difficult or impossible for many species, but where problems can be overcome rapid production of select clones (1/2 million plants from one seedling in 5 years) seems possible. Fully operational cloned planting stock production could be implemented in 10 years given 4-year field evaluation. At that stage in program development the cost of propagules has been estimated at 2 to 3 times higher than for seedlings (Brix and van den Driessche 1977). Time and cost values must be weighed against tree improvement gains through cloning. Such gains can be great.

The problems associated with the production of rooted cuttings vary greatly with species and even individual tree within species. Generally, cuttings taken from young trees in the juvenile stage root more efficiently than cuttings from older trees in the adult stage, e.g., Douglas-fir (Ross 1975), radiata pine (Libby et al. 1972), and white spruce (Rauter 1974). For some species this is not so pronounced (western hemlock,Brix and Barker 1975) and for others even the juvenile stage shows low rooting efficiency (lodgepole pine, Longman et al. 1972) or poor root form (white pine, Thomas and Riker 1950).

The rooted cuttings may grow in the field at rates comparable to seedlings (Norway spruce, Lepistö 1974), or they may achieve these rates after a lag period (western hemlock, Brix and Barker 1975), or continue to grow more slowly (radiata pine, Sweet and Wells 1974). In addition to drastic differences in growth rate, rooted cuttings may assume an irregular growth form (plagiotropism) with age of parent tree or age and position of the shoot on the parent tree, e.g., Douglas-fir (Ross 1975, Copes 1976). On the other hand, rooted cuttings from adult trees may possess some desirable adult tree characteristics such as disease resistance (Thuja plicata, Søegaard 1956).

Overall, propagation by rooted cuttings varies greatly in the rooting efficiency and growth of cuttings from species to species and from tree to tree. Furthermore, the performance of cuttings may be affected profoundly by tree age, position of the cutting on the tree, tree vigor, and the season cuttings were taken. Many of these problems can be overcome in many species by refining methodology, but the solution of the general problems summarized in figure 1 remain.

The major dilemma with forest improvement through vegetative propagation by rooted cuttings is that cuttings propagate better from juvenile trees, but selection of elite mother trees must come after they have reached the adult stage; by then propagation by cuttings becomes a severe problem (fig 1). A practice of repeated shearing of juvenile trees, "hedging", has aided in retaining the juvenile character of cuttings over a longer period (Libby and Hood 1976). Repeated cycles using rooted cuttings as the source of new cuttings, "re-rooting", has also helped to maintain juvenility (Fielding 1969). These practices may be adaptable to a variety of species but, failing that, there remains the possibility of devising more reliable selection procedures for juvenile trees or of using methods that can generate cuttings of juvenile character from adult trees (fig. 1).

Tissue Culture Propagules

The recently emerging application of tissue culture techniques to conifers provides an alternative to rooted cuttings for clonal forest propagation. Present tissue culture techniques can provide rooted propagules of forest species from multiple adventitious buds formed on embryos or on embryo and seedling parts cultured *in vitro* (Mott *et al.* 1977 and references there cited). The present tissue culture capabilities are similar to those of the rooted cutting methods (fig. 1) in that rooted propagules may be produced in a short time from juvenile seedling material. Reliable and routine rooting of the shoots produced in culture, on the scale needed in operational planting, is difficult to achieve. Cost per tissue culture propagule is high and the propagules are just beginning to be field tested and are not ready for operational planting.

The only published data on propagule production from improved tree genotypes (Mott *et al.* 1977) shows that multiple adventitious shoots can be obtained from nearly all seedling progeny of select loblolly pine trees from piedmont or coastal plain sites. An average of 20 shoots per clone can be expected on the first cycle, but methods to root a high percentage of these shoots reliably and on a production scale are lacking. As much as 70 percent rooting of these shoots has been achieved in some experiments, but production of 3-10-rooted individuals per clone is more common. Work is continuing in the laboratory (Mehra-Palta *et al.* 1977) and elsewhere to improve the rooting efficiency.

The early growth and form in soil of such propagules compare favorably with those of seedlings, and clones of few individuals can be tested simultaneously on several sites, thus multiplying the information to be gained on genotype/environment interactions during the field tests. In this way the clones can aid efforts to develop reliable methods for selecting elite trees while they are still juveniles.

Figure 1.--Generalized outline of problem areas in the propagation of elite clones of conifers by means of rooted cuttings.

Possible improvements	Source of cuttings	Rooting	Propagule behavior in field
Increase selection efficiency for young trees	Juvenile Trees (poor clone selection at juvenile stage)	+ good efficiency - poor root form - variable with tree - season dependent	+ favorable comparison with seedling
Prolong juvenile phase (hedging; re-rooting)	↓		
Reversion from adult to juvenile phase	↓		
Devise better means of rooting adult tree cuttings	Adult Trees (better clone selection at adult stage)	- poor efficiency: age/size of tree, position on tree - poor root form - inconsistent rooting - season dependent	+ adult wood qualities + disease resistance - loss of juvenile growth - plagiotropism - abnormal growth

Obviously, the tissue culture methods are at a much earlier stage of development than rooted cuttings. But all indications are that the problems in rooting the adventitious buds from tissue culture will be resolved. Furthermore, there are similarities between the two technologies that might help to resolve some of the problems encountered with cuttings. Cuttings must be studied intact because they rely on photosynthesis and shoot growth to support root initiation. Consequently, subtle influence of the mother tree on the shoot growth behavior of cuttings is inherent in the rooting process. Tissue culture procedures offer a more tractable system for the study of the controlling factors in rooting. Carbon source, nutrients, and growth regulators are supplied in the culture medium and thus buds, shoots, stems, etc. can be excised and cultured separately to discover the influence of each on the rooting process. For example, growth regulators normally produced within the plant can be supplied exogenously in an effort to mimic the contribution made by the young shoot tip that has been removed. Finally, the more extensive information on growth behavior of rooted cuttings may point the way for tissue culture studies.

Some preliminary data from studies of basic rooting factors are summarized in table 1. Rooting efficiency in response to the growth regulators NAA (α-napthaleneacetic acid) and BAP (6-benzylaminopurine) is compared for seedlings lacking shoot and root -- "hypocotyl sections"; seedlings lacking roots -- "derooted seedlings"; the terminal growing bud of seedlings -- "excised seedling shoots", and for similar "adventitious buds" produced in culture. The rooting efficiency is affected by other factors including the age of the seedling and the culture environment, etc. These effects are reflected in the range of percentage rooting given for each growth regulator concentration in table 1.

Table 1.--Summary of *in vitro* rooting efficiency to be expected for different seedling parts and tissue culture buds in response to growth regulator stimuli

Growth regulator concentration (mg/l) supplied in agar medium		Percent rooting			
NAA	BAP	Excised hypocotyl sections	Derooted seedlings	Excised seedling shoots	Adventitious tissue culture buds
0	0	0-6	0-37	0	0
0.1	0	0-27	0-12	--	20-30
0.1	0.1	0-27	80-90	70-80	40-70
0	0.1	0	0-12	0	0

Experiments of this type suggest that different exogenous growth regulator supplies are needed depending on which parts have been excised. Further experiments should define corrective measures to be taken when certain parts of seedlings or shoots are absent or functioning improperly. This information should lead to more efficient methods for the rooting of cuttings from trees. For example, the poor rooting of cuttings taken from adult trees might be corrected by suitable treatments to improve the vigor of the shoot bud on such cuttings.

Buds of loblolly pine produced by tissue culture are adventitious and possess juvenile character. The adventitious buds may be obtained from seedling hypocotyl and stem sections and the adventitious buds themselves are capable of successive rounds of new adventitious bud formation. The juvenile character of adventitious buds so produced holds great promise for vegetative propagation of adult trees. Procedures are now being tested for adventitious bud production from cultured parts of older trees. These buds develop on parts cultured after removal from influence of the adult mother tree, so they can be expected to revert to juvenile character. Once established, the adventitious buds could be used in successive rounds of propagule generations in the short time and small space characteristic of tissue culture propagation of herbaceous plants (Murashige 1974). This would, of course, permit vegetative propagation of elite trees selected in the adult stage (fig.1).

Adventitious buds can be obtained from continuously subcultured, disorganized callus cultures of aspen but there is evidence that clones produced in this way may not be uniform (Lester and Burbee 1977) and this might argue for bud production from excised tree parts rather than from subcultured callus.

Propagule Evaluation *in vitro*

It seems certain that the methods for rapid vegetative propagation of conifers on a production scale will soon be at hand. The capacity of rapid propagation brings with it the need for early and efficient testing for such things as disease and pest resistance. An elite adult tree may have merely escaped certain diseases in its present site; the propagules may present a substantial hazard for other sites. Early selection for resistance in the laboratory on cultured tissues would permit tests for disease resistance to keep pace with potential propagule production. Tissue culture may also provide a more tractable system for study of mechanisms of disease resistance, similar to the approach outlined earlier for rooting.

We have succeeded in rearing southern pine beetle from eggs on a loblolly pine callus substrate (Mott and Thomas 1977). This system can now be used to study pest/host interactions and host resistance. Fusiform rust resistance of young loblolly and slash pine seedlings and

excised seedling parts can be identified in culture and the rust may also be cultured axenically (Amerson and Mott, in press). This opens the way for pathogen/host interactions and disease resistance mechanisms to be studied on this and other important diseases under controlled in vitro conditions.

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MASS PRODUCTION ALTERNATIVES FOR FAST-GROWING SPRUCE HYBRIDS

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ABSTRACT.--A reciptrocal crossing program of Picea glauca and P. omorika, and P. mariana and P. omorika with adequate intraspecific control crosses was carried out. Yields of full seed/cone ranged from 0 to 2.6 for the P. glauca-P. omorika combination. These low seed sets and a 9-day difference in female receptivity rule out producing this hybrid via seed. The full seed/cone yields of the P. mariana-P. omorika combinations averaged approximately half the yield of the intraspecific crosses. However, receptivity of the two species is well synchronized and in spite of reduced yields, mass production via seed is a practical option. A seed orchard design is outlined and a 4-acre grafted orchard may produce at least 75,000 plantable seedlings on 6- to 8-year-old grafts. Results of a rooting trial of selected hybrid seedlings are described. A practical mass production program for the P. glauca-P. omorika combination based on rooted cuttings is described. The program would be based on juvenile selection among 4-year-old seedlings, cloning, and the simultaneous establishment of clonal trials and hedges. The hedges would be a source of cuttings for future mass production--a hedge 400 feet long by 5 feet high would produce a minimum of 20,000 cuttings annually.

In 1959 a single cross between native Picea mariana and P. omorika was produced at Rhinelander, Wisconsin. Seed set was high and height growth of the hybrid was superior to the native species.

A P. omorika x P. glauca cross was first produced in 1964. In the greenhouse, mean early height growth of the hybrid exceeded the growth of P. glauca by 45 percent, and in 1975 in a field test exceeded the open-pollinated progeny of the P. glauca by 14 percent. The 1964 control cross P. omorika x P. omorika produced 9.8 full seeds per cone; in comparison the interspecific cross yielded 7.4

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seeds/cone. Both values are low, because up to 60 to 80 filled seeds per cone have resulted from other P. omorika x P. omorika crosses. Comparison among the 1964 crosses, however, suggested that the two species were cross compatible and that P. omorika x P. glauca crosses generally would result in high seed yields.

In 1971 both crosses were repeated on a larger scale with adequate controls to verify the superior growth of the hybrids and to determine the potential for mass production by seed. This trial clearly showed that P. omorika x P. mariana can be readily produced by seed on a large scale but that other means of reproduction must be found for the P. omorika x P. glauca hybrid. In 1975 a study was begun to test the possibility of vegetatively propagating P. omorika x P. glauca by means of cuttings.

It is the purpose of this paper to compare the sexual mass production with the vegetative mass propagation of these two promising spruce hybrids. The emphasis will be on propagation potential; performance of the hybrids will not be discussed.

SEXUAL MASS PRODUCTION OF HYBRIDS

Methods

Three individuals of each of P. glauca, P. omorika, and P. mariana were selected for vigor and the combinations P. glauca-P. omorika and P. mariana-P. omorika were tested. Intraspecific crosses were used as controls.

The selected P. glauca parents have been included in many tests to date and their progenies have consistently been top performers. The 1971 pollinations were made on 1959 grafts growing near Lake Tomahawk, Wisconsin.

P. mariana and P. omorika selections were made in a 1936 planting of spruce species growing on an upland site in Forest County, Wisconsin. Vigor of the trees was the only selection criterion.

The crossing scheme, including reciprocals, should have yielded the following number of crosses:

Combination	No. of crosses
glauca - omorika	18
mariana - omorika	18
glauca - intraspecific	6
mariana - intraspecific	6
omorika - intraspecific	6
	54

Due to lack of female conelets, particularly on P. omorika, only 44 of the crosses were attempted and of those 42 actually produced seed. In addition the three P. omorika trees were used as male parents in six crosses with two additional P. omorika genotypes and the three P. glauca genotypes were crossed with a random mixture of P. glauca pollen.

For P. glauca and P. mariana, fresh 1971 pollen collections extracted and cleaned at the laboratory were used. Pollen collected in 1970 and stored under various conditions were used for P. omorika crosses on P. glauca and on the two P. omorika genotypes. However, because no meaningful storage effects could be identified on either inter- or intraspecific crosses, the seed yield data were combined. A mixture of pollen from the various storage conditions was used to pollinate the three P. omorika and the three P. mariana trees in the Forest County, Wisconsin, test plantation.

Bagging of P. glauca was on May 10, 1971, and the later-developing species were bagged when the female strobili could be recognized. The following schedule shows the time of pollinations, debagging, and cone harvest:

♀ Species	Pollination	Debagging	Cone Harvest
		1971 Date	
P. glauca	5/21	6/4 - 6/8	9/1
P. omorika	5/30	6/11	9/21
P. mariana	5/30	6/11	9/9 - 9/21

For each pollination bag harvested, cones were counted and each individual cone extracted by hand to assure that all seeds were extracted. After dewinging and cleaning, the total sample was X-rayed

and the number of full seeds counted on the X-radiographs. Last, the total number of seeds was sampled from eight bags on each of the three P. glauca and two to three bags in the case of P. mariana and P. omorika. From these counts the average total number of seed and full seed per cone were determined.

Unpollinated control bags were placed on all trees and only one bag on P. omorika yielded seed.

Results and Discussion

Seed yields in intraspecific crosses

The three P. glauca crosses (two diallels and one with mixed pollen) averaged 34, 43, and 47 full seeds per cone (for 1885, 1887, and 1889, respectively). These values compare favorably with previously published values for the same clones (Nienstaedt and Jeffers 1970, King et al. 1970). For individual crosses, yield of full seed ranged from 22 to as high as 73 (table 1). The latter value is higher than any previously published (Nienstaedt 1972). The results of the individual crosses suggest that clone 1889 is more productive than either 1885 or 1887; this substantiates previously published results (Nienstaedt and Jeffers 1970, King et al. 1970).

In the three P. mariana trees the two diallel crosses per tree averaged 27, 38, and 14 full seeds per cone for trees 5343, 5344, and 5340 respectively, and ranged from 11 to 38 full seeds per cone for individual crosses (table 1). These results are good because P. mariana cones usually average 43 full seeds per cone.

Table 1.--Yield of full seed from diallel crosses of three selected trees

Picea glauca

Female parents	Male parents: 1885	1887	1889	Random mix
1885	0 [2/]	32 / *127*	37 / *83*	23 / *88*
1887	22 / *29* [1/]	0	54 / *54*	39 / *18*
1889	58 / *38*	42 / *79*	0	73 / *37*

Picea mariana

Female parents	Male parents: 5343	5344	5340
5343	0	27 / *78*	27 / *90*
5344	38 / *75*	0	38 / *99*
5340	17 / *36*	11 / *36*	0

Picea omorika

Female parents	Male parents: 5342	5347	5341
5342	0	46 / *39*	74 / *22*
5347	0	0	0
5341	68 / *4*	78 / *2*	0

1/ 22 / *29* = No. of full seed per cone. *No. of cones harvested.*

2/ 0 = Cross not performed.

The sample of 20 cones from the three P. omorika trees averaged a total of 87 seed per cone. The diallel crosses were only completed on 2 of the 3 trees and on the average yielded 56 and 71 full seed per cone on trees 5342 and 5341, respectively. The overall average was 57 full seed per cone. For individual crosses, yields ranged from 46 to 78 full seed per cone (table 1). This also shows that pollen quality and timing of pollination was good.

Seed yields in interspecific crosses

Contrary to expectation, the crosses between P. glauca and P. omorika yielded little or no seed regardless of the direction of the cross (tables 2 and 3).

Table 2.--Yield of full seed from diallel interspecific spruce crosses

Picea glauca and Picea omorika

Female parents of Picea glauca	Male parents Picea omorika 5342	5347	5341	Female parents of Picea omorika	Male parents Picea glauca 1885	1887	1889
1885	0.06[1] / *839*	0.04 / *615*	0.11 / *666*	5342	0.68 / *57*	2.61 / *31*	0.43 / *46*
1887	.14 / *503*	.01 / *272*	.15 / *154*	5347	.00 / *7*	0[2]	0
1889	.04 / *479*	.04 / *292*	.05 / *756*	5341	1.67 / *3*	2.33 / *3*	.00 / *1*

Picea mariana and Picea omorika

Female parents of Picea mariana	Male parents Picea omorika 5342	5347	5341	Female parents of Picea omorika	Male parents Picea mariana 5343	5344	5340
5343	11 / *99*	13 / *70*	22 / *140*	5342	49 / *13*	44 / *49*	74 / *4*
5344	14 / *42*	18 / *70*	21 / *72*	5347	0	0	0
5340	5 / *44*	5 / *53*	7 / *26*	5341	0	0	0

[1] .11 = No. of full seed per cone.
666 = No. of cones harvested.

[2] 0 = Cross not performed.

Table 3.--Average yields of full seed/cone from diallel intra- and interspecific spruce crosses

Cross combination	Cones	Full seed	Ave. full seed/cone	Ave. seed/cone[1/]	Full Seed
			-Number-		Percent
P. omorika x P. omorika	256	7,695	30.1	86.67	35
P. omorika x P. glauca	66	3,090	46.8	90.55[3/]	52
P. mariana x P. mariana	414	12,130	29.3	43.03[2/]	68
P. mariana x P. omorika	612	8,907	14.6	43.03[2/]	34
P. omorika x P. glauca	148	152	1.0	86.67	1
P. glauca x P. glauca	553	21,564	39.0	71.33	55
P. glauca x P. omorika	4,576	312	.07	71.33	.1

[1/] Unless footnoted, the derivation of these values is explained in the text.

[2/] Based on counts of all bags with P. mariana x P. mariana crosses -- a total of 414 cones.

[3/] Based on counts of 11 cones from tree 5342 -- the only tree in the cross.

Crosses between P. mariana and P. omorika, on the other hand, were successful. The average yield of full seed per cone when P. mariana was the female parent was 14.6 seed, about half the amount of seed obtained in the intraspecific P. mariana crosses (table 3). Mean full seed per cone yields of individual mother trees were 16, 18, and 5 seeds per cone for trees 5343, 5344, and 5340 as compared to the 27, 38, and 14 full seeds obtained from the intraspecific crosses. Individual crosses yielded from 5 to 22 full seeds per cone (table 2). Lowest yields were on tree 5340, but the cones on this tree contain only 19 seeds per cone as compared to 43 for 5343 and 53 for 5344. Nevertheless, 5340 appears to be the least cross compatible of the three trees--full seed yield was 27 percent of total seed yield as compared to 37 and 34 percent for the other trees. Unfortunately, it was only possible to perform the P. mariana crosses on one of the P. omorika trees--5342. This tree is characterized by a total yield of 91 seeds per cone and yielded an average of 56 full seeds per cone in intra-specific crosses--62 percent of total seed set. The seed yield for P. mariana crosses was almost as good (tables 2 and 3) averaged 47 seeds per cone (52 percent of total seed set).

Discussion

The above yields of seed clearly show that sexual mass production of hybrids between P. glauca and P. omorika is impractical. The 9-day difference in the phenology of the two species would necessitate an artificial pollination technique. But even if a practical mass pollination technique could be developed, the low seed yield would present an insurmountable obstacle.

It is, on the other hand, feasible to sexually mass produce hybrids between P. omorika and P. mariana. Phenologically, the species are well synchronized and even though the seed sets are reduced, they are still substantial.

The three crosses on the P. omorika parent (5342) suggest that it would be best to use P. omorika as the female. Other evidence supports this conclusion. In 1960 seed was collected from nine P. omorika trees growing near Eagle River, Wisconsin. Plots of P. mariana are located close by in the same test.[2/] The progenies were planted in 1965 near Lake Tomahawk. In 1975, 129 of the original 180 trees had survived and of the survivors, 86 were identified as hybrids between P. omorika and P. mariana. Allowing for differential survival, we estimate that between 50 and 60 percent of the original trees were of hybrid origin. Hybrids were found in every progeny and must have represented from about 30 to as much as 90 percent of the trees in individual families. Thus, P. omorika 5342 isn't an isolated case of high cross compatibility between the two species. All the nine trees in the progeny test must have been phenologically well synchronized with the adjacent P. mariana and generally are cross compatible.

VEGETATIVE MASS PRODUCTION OF HYBRIDS

Vegetative propagation has been suggested as an alternative to sexual propagation of cross incompatible species combinations (Roulund 1971)and several schemes have been suggested for mass propagation of juvenile selections of various Picea species (Rauter 1974, Kleinschmit 1974, Kleinschmit et al. 1973, and Roulund 1976).

King et al. (1965) have shown that juvenile selections in 2-2 P. glauca retained superiority 7 years after field planting; more recent measurements at 22 years have shown that the selected seedlings are still 30 percent taller than the control trees.

In 1974 a study of selected juvenile P. glauca mass propagation by cuttings was begun and in 1975 it was extended to include the hybrid progenies described in the previous section.

2/ The P. omorika and P. mariana parents of the crosses were from the same tests.

Source of Material

The seed was cleaned by blowing and for some samples by submerging in 95 percent ethanol to remove empty seed. Seeds were sown in perlite in glass-covered stainless steel trays on Jan. 29 and 30, 1973. The trays were kept in the greenhouse at 20-25°C and received 18-20 hours of light each day. The major period of transplanting was February to March, but lower than expected germination and unexplained seedling mortality necessitated resowing and replacement of some progenies in early May. Seedlings were transplanted into Ontario 5/8 inch tubes in a 1:1:1 sand-soil-peat mixture. In August the seedlings were transplanted to the nursery. Shade was provided initially.

The tallest 104 seedlings were selected during the summer of 1976. They represented all inter- as well as intraspecific combinations except *P. omorika* x *P. omorika*. In addition, six *P. glauca* and seven *P. mariana* seedlings of average heights were selected as controls. The mean height superiority of the selected seedlings is expressed by the following values:

Parent combination	Mean ht. superiority over mean ht. of control
	percent
P. glauca x *P. glauca*	103
P. glauca x *P. omorika*	78
P. mariana x *P. mariana*	39
P. mariana x *P. omorika*	52

Cuttings were taken from the plants with pruning shears. They were 7-1/2-to 9-cm-long first and second order branch terminals. The top whorl was too coarse to be used. Cuttings were immediately put in plastic bags on ice in a cooler and within 24 hours were placed in the rooting bench.

Rooting environment

Rooting was on an open greenhouse bench provided with an automatic mist system, bottom heat, and an incandescent floodlight system to extend the photoperiod.

Air-temperature was set at 21°C (70°F) but substantially exceeded this on sunny days.

Media temperature was set at 26 to 29°C (79 to 84°F) but reached 32° (90°F) on sunny days, which damaged the cuttings.

Mist was applied for 15 seconds every 15 minutes during the day and 15 seconds every 30 minutes during the night.

The aim was to keep the cuttings constantly wet without excessive run-off but on sunny days the tops of the cuttings were dry on part of the bench. Also, because the water was hard, it covered cuttings with salts.

Media was gravel - peat - medium grade perlite, 1:1:1. Gravel was obtained by sifting pit run sand through 1/8-inch hardware cloth. pH was 4.0 initially and gradually increased to 6.0, a more favorable level.

Light regime was 19 hours photoperiod.

Fertilizer was applied weekly after rooting by drenching with Peter's Fertilizer (20-20-20) at a 1:128 ratio.

Cooling-off period consisted of reducing mist to 15 seconds every 30 minutes during the day and 15 seconds every 45 minutes during the night.

Newly transplanted cuttings were also kept under mist for a short period of time.

Experimental design

Containers were "Fives" rootrainers by Spencer-LeMaire[3/]. One book of five cavities constituted a plot. Plots were arranged in five randomized complete blocks on a greenhouse bench.

Rooting schedule

September 27 and 28, 1976	Collection of cuttings
September 28 and 29, 1976	Sticking of cuttings
December 6, 1976	Cooling off period begun
December 23, 1976	Fertilizer treatment begun
January 7 to 26, 1977	Root evaluation and transplanting to 4 inch clay pots.

Results and Discussion

Rooting of clones of intraspecific crosses and control seedlings

In total, we rooted 16 clones of P. glauca (10 from selected seedlings and 6 from controls) and 29 clones of P. mariana (22 selected for vigor and 7 controls).

3/ Mention of trade names does not constitute endorsement of the products by the USDA Forest Service.

The mean rooting percent for selected P. glauca and P. mariana clones was close to 50 percent. P. glauca controls did not root as well (42 percent) as the P. mariana controls (63 percent) (table 4).

Table 4.--Cuttings rooted from selected Picea glauca and P. mariana clones
(In percent)

Picea glauca

Female parent	Male parent 1885	1887	1889
1885		40.0 (3 1/)	48.0 (1)
1887			54.0 (2)
1889	54.0 (4)		

All 10 clones $\bar{x}$ = 49 percent

Control
4885 2/ 42.0 (6)

Picea mariana

Female parent	Male parent 5340	5343	5344
5340			58.7 (3)
5343	48.0 (4)		
5344	49.0 (8)		

All 15 clones $\bar{x}$ = 51 percent

Select
7169 3/ 49.1 (7)

Control
7169 2/ 62.9 (7)

1/ *Number of clones included in the mean values.*

2/ Open pollinated unselected control clones.

3/ Open pollinated seedlings selected for vigor and cloned.

Variation among individual clones was large and statistically significant. P. glauca rooting percentages ranged from 20 to 88 for selected clones and from 8 to 76 for control clones. Comparable values for P. mariana were 16 to 80 and 44 to 76 percent for selected and control clones, respectively. Rooting equaled or exceeded 60 percent in 30 percent of the selected P. glauca and 36 percent of the selected P. mariana clones.

Rooting of clones of interspecific crosses

Mean rooting of four P. glauca x P. omorika and five clones of

reciptrocal crosses was 67 and 56 percent, respectively. For individual clones in the two groups, rooting percentages range from 36 to 92 and from 36 to 68 percent, respectively (table 5). The data suggest that the hybrids are better rooters than P. glauca crosses and controls, but the differences are not statistically significant. The number of roots per cutting showed a similar and significant trend.

Table 5.--Cuttings rooted from selected clones of hybrids of Picea omorika, P. glauca, and P. mariana

(In percent)

P. glauca and P. omorika

Female parent of Picea glauca	Male parent of Picea omorika			Female parent of Picea omorika	Male parent of Picea glauca		
	5342	5347	5341		1885	1887	1889
1885				5342		52 2	
1887		92 1 1/	70 2	5347			
1889			36 1	5341		59 3	
All 4 clones $\bar{x}$ = 67				All 5 clones $\bar{x}$ = 56			

P. mariana and P. omorika

Female parent of Picea mariana	Male parent of Picea omorika			Female parent of Picea omorika	Male parent of Picea mariana		
	5342	5347	5341		5343	5344	5340
5343	62 5 1/	76 1	74 2	5342	65 3	55 11	65 28
5344	48 2	47 3	48 6	5347			
5340	50 2			5341			
All 21 clones $\bar{x}$ = 55				All 42 clones $\bar{x}$ = 63			

1/ *Number of clones included in the mean values.*

Overall, the rooting of the clones of the P. mariana and P. omorika combinations were comparable. Forty-two P. omorika x P. mariana clones had a mean rooting value of 63 percent and 21 clones of the reciprocal combination had a mean rooting value of 55 percent. The ranges in rooting percentages within these two groups were large; from 36 to 96 percent for the crosses in which P. omorika was the female parent and from 12 to 84 percent when the mother was P. mariana. The data in table 5 suggest that the ability to root is under strong genetic control; however, although the differences in rooting percent of individual clones were highly significant, the analysis of variance could not demonstrate significant parent tree effects. The number of roots per cutting was also determined and for this characteristic parent tree as well as clonal effects were significant.

Discussion

Because the environmental control was inadequate, rooting was less than anticipated for juvenile material. Two blocks on one end of the greenhouse bench had particularly poor rooting success. Therefore, the rooting in the three other blocks may be a better indication of the rooting potential of the clones. As an example, for all 5 blocks the rooting in 52 (47 percent) of the clones equaled or exceeded 60 percent. In the 3 better blocks, 82 (75 percent) of the clones attained this level of rooting. It is likely that even better rooting can be achieved if optimum conditions could be attained for an entire test (Kleinschmit 1974).

Mass production is only practical if large numbers of propagules can be produced for an extended period of time. But time connotes aging and rooting ability declines as stands mature. Hedging may be one answer to the problem (Libby 1974).

CONCLUSION

P. omorika x P. mariana seed production will require grafted seed orchards. Two orchard designs are possible:

(1) A simple two clone design -- in such an orchard, seed would be harvested on both the P. omorika and the P. mariana clone and, except for a small portion of selfed seed, would all be expected to be of hybrid origin.

(2) Orchards of one P. omorika clone and several P. mariana clones--hybrid seed would be harvested only on P. omorika. Whether or not the seed on P. mariana would be harvested would depend on the intraspecific general combining ability of the P. mariana clones. Good combiners could yield improved P. mariana seed. A small admixture of hybrid seed could probably be expected, but should not deter from the quality of the material.

The performance of the progenies involved in the studies I have discussed indicate the desirability of control-pollinated progeny testing before or concurrent with seed orchard establishment. Only three parents of either species were tested, but heights of progenies clearly showed that one P. mariana was a superior general combiner. This consideration may favor the orchard with many P. mariana clones. Inferior clones could be rogued on the basis of progeny test results.

Early productivity of seed orchards can perhaps be roughly estimated. P. omorika is not a prolific cone producer, but it might produce 10 cones per ramet at 6- to 8-year from grafting. With this assumption, a 4-acre orchard with 1,250 grafts (10 x 12 foot spacing)--250 P. omorika and 1,000 P. mariana (20 clones of 50 ramets each)--would produce 117,000 full seed (46.8/cone, table 3) which may yield approximately 75,000 plantable seedlings.

P. glauca x P. omorika or reciprocal hybrids would have to be vegetatively mass produced. A practical program may be as follows:

(1) Use 20 to 30 select P. glauca and 5 to 10 select P. omorika to produce 150 to 200 crosses between the two species. The majority of crosses should be on P. glauca and each cross should involve a minimum of 500 conelets. A program of this magnitude would probably produce from 3,000 to 6,000 hybrid seed.

(2) Fall sow in greenhouse and spring plant in the nursery at 18 x 18 inch spacing. If enough seedlings are available, an adequate statistical design should be used.

(3) At the end of the fourth season in the nursery, select the best 6 seedlings in the best 20 families.

These 120 seedlings will form the basis for the following clonal testing and mass propagation scheme:

First year. In the fall, stick 40 cuttings per clone. Discard all clones with less than 25 rooted cuttings.

Second year. In the fall, plant

(1) short-term clonal nursery test using single tree plots with 15 replications.

(2) at least 10 ramets for each clone selected on the basis of rooting, in double-row hedges with 1 foot between plants and 1 foot between rows (hedges would be 8 feet from center to center).

Third year. In the fall, stick 75 cuttings per clone--3 from each ramet in hedge as well as test.

The program in subsequent years may either seek the earliest possible production of stock for field planting or may aim at rapid build-up of the hedges. The clones will be evaluated at 5 years after the establishment of the test (7 years from the beginning of the program).

During the same period, it should be possible to establish 400 to 500 feet of double row hedges of each clone. If five cuttings could be produced per square foot of hedge, a 400-foot hedge 5 feet high would produce 20,000 cuttings, which would be 12,000 to 16,000 plants for field planting annually.

The productivity of the total program would, or course, depend on the final number of clones included in the hedge collection. If 30 clones of the original 120 were retained, we could expect from 360,000 to 480,000 plants from approximately 2-1/4 acres of hedges.

The program as outlined will be time-consuming and probably involve a minimum of selections. It could readily be increased without increase in the rooting facility if new clones were added each year by winter propagation. Profitability will depend on the gains in growth and frost resistance.

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IMPROVED TREES: ECONOMIC PROMISES AND PITFALLS

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ABSTRACT.--Surging demands for wood fiber that must be produced on a decreasing acreage of forest land suggest soaring prices, a shrinking market for wood products, or both. Either of these consequences can be forestalled or prevented by implementing existing technologies, one of which is cultivation of genetically improved trees. Multiple and sizable gains from improved trees are likely. Gains are not without risks, however, as possible losses may occur through failure to accumulate capital necessary to establish plantations of improved trees on good sites.

Reasons for widespread reliance on improved trees are plentiful and pressing. Almost daily, trade announcements and public media forewarn of escalating demands for wood fiber from a diminishing land base. Like a mathematical equation, the oft reported and most assured result is a shortfall in timber supplies, signaled initially by soaring prices, and quickly followed by deteriorating markets as competing materials replace wood. Whether these calamitous outcomes are forthcoming is a matter of speculation, but the ability of improved trees to ameliorate such dire consequences is unquestioned. As a means to increase and improve the nation's wood supply, the genetically improved tree has no peers.

Yet, use of genetically improved trees is not without risk. In this paper, I emphasize probable gains and economic promise, but also describe hazards. I also review a method for analyzing the economics of tree improvement recently advocated by Clark Row and me.

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MORE WOOD FROM FEWER ACRES

A program to provide improved planting stock calls for substantial investments of money, land, and labor. All these productive resources could be profitably used elsewhere; they remain in timber production only as long as rewards are high enough to keep them there. Strong and stable demands for wood for the foreseeable future underlie decisions to commit resources to timber production. Thus, it behooves us to briefly examine the probable strength and durability of the demand for wood.

Most experts anticipate an upward climb in the demand for wood fiber (Armitage 1976, Zobel 1977b). Consumption of wood by the entire world is expected to rise from 95 billion cubic feet in 1975 to 140 billion cubic feet in 1990, an increase of approximately 50 percent in only 15 years (Armitage 1976). Worldwide pulpwood consumption is projected to rise even faster, doubling by 1985. Reports published by the USDA Forest Service project steep increases in consumption for industrialized nations, especially the United States, Japan, and countries in western Europe (Phelps 1975).

A significant hike in the supply of timber could prevent price increases, but even optimists are projecting, at best, a modest increase in available wood on the world market. Thus, the present trend of rising relative prices for wood and wood products will likely extend indefinitely into the future. And, investments in tree-improvement programs will probably not be buffeted by waves of dwindling demand.

Major additions of land devoted to timber production cannot be expected. In fact, historical evidence and a parade of prognosticators assert the opposite; i.e., land devoted to commercial production of wood fiber has declined and will continue to do so. Not only is forest acreage declining, but the more productive lands often are among the earliest casualities. Many productive sites are diverted to agricultural crops as landowners seek higher profits. Rich soils in bottomlands face a similar fate or inundation. And, sites both rich and poor face limitations on commercial timber production as environmental restrictions are imposed.

The Mississippi Delta offers an example (Sternitzke and Nelson 1970). Since the earliest surveys in the 1930's, almost 40 percent of the 12 million acres of commercial forest land has been lost to other uses. Even land held by forest industries has declined because of the lure of higher profits in other land uses. Loss of quality land is underscored by the growing percentage of forest acres of species that inhabit only poorly drained clay flats. Forestry's losses were gains for soybeans, improved pastures, and cotton, in that order.

Without further belaboring the point, it appears certain that demand for wood fiber will remain strong and that timber supplies will come from a smaller land base. Such circumstances suggest that prudent investments to augment timber supplies will be profitable. One such investment of proven value is in tree improvement. Economic analyses paint an encouraging benefit--cost picture for tree-improvement programs as a means of accelerating per acre production of wood.

BENEFITS OF IMPROVED TREES

In considering the host of benefits that reputedly accrue to the new subscriber to improved trees, a critical oversight frequently occurs. Tree improvement is pictured as independent of other recommended management practices. No assumption about improved trees is more likely to reap financial havoc. Yield increases promised for genetically-improved planting stock occur only when properly combined with site selection, site preparation, fertilization, and silvicultural maintenance. Gains from improved trees, as with most intensive forest practices, demand close adherence to a series of recommended treatments. Attempts to reduce costs or cut corners in silvicultural prescriptions will likely erase gains. Benefits are listed here in the context of fully integrated forest management.

Much attention and effort in the South have been directed to pines, but hardwood tree-improvement programs are also well underway, and hardwoods promise to respond well to improvement efforts. Demand for pine stumpage, markets for pine products, relative ease with which pines can be genetically improved, and overall economy, however, have dictated some neglect of hardwoods and concentration on softwoods. Thus, most gains and analyses mentioned in this paper pertain to softwoods.

Overall objectives of tree improvement programs read like familiar sign posts: faster growth rates, increased resistance to forest pests, better tree form and wood quality, and heightened adaptability. If our storeroom of economic data were replete with necessary and accurate information, and if our markets fit the requirements of perfect competition, then our economic analyses could clearly assess costs and benefits of each stated objective. In the absence of both data and free markets, we can only roughly estimate probable gain. Nevertheless, one conclusion surfaces over and over: improved trees promise substantial financial reward at minimal cost, especially for industrial landowners. This beneficial ratio stems largely from gains in specific tree characteristics which individually and collectively enhance the value of the wood for processing. Furthermore, small per-acre gains in desirable characteristics become significantly more important when measured over large tracts of managed forest lands.

Volume gain is a primary goal for most tree-improvement programs. Early advocates of genetic improvement predicted volume gains in excess

of 50 percent, but current workers expect volume increments of 10 to 20 percent in the first breeding cycle (Zobel 1977a). Additional gains are expected in future generations, but a gain of 10 to 20 percent appears reliable and defensible.

Tree straightness is desirable, but not easily measured. Straight trees more than pay for themselves when accountants measure harvesting and manufacturing cost advantages and the increased yield as sawtimber is produced. Geneticists report, too, that this goal can be achieved quickly, possibly within one generation of breeding (Zobel 1974).

Another characteristic desired in the South is resistance to fusiform rust. Every analysis stresses importance of progress toward this goal. If trees die, growth and conversion economics become meaningless. But resistant strains are being found and made available for planting in the South (Zobel 1974). Specific gravity of wood can be a significant determinant of pulp yield and processing time in the mill. Moreover, specific gravity increases of 5 to 7 percent have been realized in tree-improvement programs (Ottens and Carlisle 1976).

Revenue gains from tree-improvement programs are measured by comparing additional costs and returns associated with improved planting stock. Only costs or gains over and above those for plantations of unimproved trees are relevant. In practice, additional costs are minimal; in most instances, the only additional or marginal cost goes for improved seeds. Since the economic analysis compares the extra seed costs with discounted values of extra yields at harvest, added expenditures for improved trees are strongly supported. Yield increases of about 5 percent more than justify foreseeable expenditures for seeds (Zobel 1974). Ottens and Carlisle (1976) found that seed costs could be 10 times greater than current rates and still promise profits over a typical rotation. Similarly, it was shown that discounted gains in revenue exceeded current costs of seed production by a multiplicative factor of 4 to 70, depending upon price and yield assumption. In every case, results implied an underinvestment in tree improvement.

Other economic analyses focused on present net worth or internal rate of return as criteria for evaluating tree improvement. Except at abnormally high discount rates, present net worth of investments in tree improvement proved profitable (Dutrow and Row 1976). Internal rates of return were favorable too. Most studies reported rates exceeding 10 percent, ranging up to about 20 percent (Dutrow and Row 1976, Porterfield 1974). Profits from tree improvement can be traced to nominal cost increments even more than to value gains. The cost of improved trees is only a few dollars more than normal stock, and this cost is incurred only once per rotation Other cost advantages should not be overlooked. For example, acres of forest lands required to supply a mill can be reduced, with associated economies in transportation, management, and harvesting operations. From a national or regional

perspective, tree-improvement programs release forested acres for other uses by reducing land required to supply a given amount of wood.

Although results and recommendations are similar, a recent study by Clark Row and myself warrants discussion before we consider the possibility that our chosen path may lead to economic pitfalls (Dutrow and Row 1976).

In our approach, we tried to address the question that haunts economists' efforts to predict dollar returns from improved trees: what is the relative growth pattern of superior trees during an entire rotation? Do superior trees exhibit an early burst of growth and then merely parallel growth rates of normal planting stock? Or, is the early growth advantage of improved trees maintained throughout rotations? Admittedly, these questions will be answered only by time. Our technique offers an improved estimate in the interim.

Equations were derived from data on height, diameter, volume, and survival of unimproved loblolly plantations, and were then modified to show gains made by 8-year-old trees in actual tree improvement programs. Indices of gain were then calculated and inserted in the growth equations for unimproved loblolly plantations to simulate behavior of genetically-improved stock in a plantation environment. With growth equations modified to reflect genetic gain, we were able to estimate corresponding economic gains.

Some of the specific measures of gains through use of improved loblolly pine trees are of interest. Height indices or adjustment ratios were calculated and reflected an average gain of 3.5 percent over unimproved stock. In over 50 percent of the genetic trials height gains were at least 2.6 percent, and gains of more than 10 percent were recorded for 17 percent of the trials.

Diameter-adjustment ratios were derived by dividing the average diameter of genetically improved trees by the average diameter of unimproved trees. Tree gains were converted to stand gains by applying the diameter-adjustment ratio to anticipated maximum and mimimum diameters; relative frequencies of basal areas occurring for the enlarged diameters were also calculated. The simulation was run, and notable gains in diameter occurred. Fifty-four percent of the genetic crosses showed diameter growth that surpassed the unimproved trees. Average growth increases were about 2 percent, with almost 20 percent of the genetic crosses exceeding a 10 percent gain over unimproved stock. Survival gains also were estimated and results were similar to gains in height and diameter. Volume estimates, however, required a slight departure from the foregoing procedures.

Volume gains in juvenile trees are not directly proportional to volumes anticipated at harvest for either improved or unimproved trees. Thus, instead of calculating "volume-adjustment factors", we estimated diameters and heights at harvest and inserted these measures into accepted volume equations. Average volume increases were significant, about 12 percent per acre. Over large tracts, 63 percent of the acres of improved trees would exhibit growth rates higher than acres of unimproved trees. Furthermore, 20 percent of these acres of improved trees would display volume gains of more than 30 percent.

As in any economic analysis, we eventually turned to evaluating financial implications of gains expected from improved trees. Present net worth was one of the criteria we used for comparing improved and unimproved trees. About 6 percent of the genetic crosses showed financial gains of up to 150 percent. The average increase in present net worth was almost 30 percent, with a median value of 16 percent. Very few of the genetic crosses indicated returns poorer than unimproved trees.

The estimates can be transformed directly from percentage to dollar gains for private plantation managers. Industrial landowners can expect nearly a 30 percent increase in present net worth. For example, if a forest manager estimates a net worth of his loblolly plantation at $100 per acre, he can increase the estimate by about 30 percent or $30 if he changes to genetically-superior stock. Or, stated differently, he could afford to pay up to $30 more per acre for genetically-improved seedlings.

For large timber-producing regions or ownerships with substantial acreages, gains from tree improvement justify large expenditures in nurseries, orchards, and research to assure even higher productivity. For example, in the three-state area of South Carolina, Georgia, and Florida, loblolly pine plantations occupy about 1,100,000 acres. If through conversion to genetically-improved trees, financial productivity per acre could be increased by 30 percent, extremely large investments in tree improvement efforts, with associated economies of scale, would be justified. Since the forest land base is shrinking, publicly subsidized tree-improvement efforts would enhance wood supplies on those acres retained for commercial production.

POTENTIAL PITFALLS

Before the list of benefits from tree improvement generates absolute confidence in our abilities to meet future wood demands, we need to recognize a few pitfalls. Some financial dangers are commonly recognized, but repetition can do no harm. Other dangers are not so well known, yet cast threatening shadows along future paths of tree-improvement efforts.

A familiar and oft cited note of caution is that improper site selection can erase all promise of gain and cause financial ruin. In most cases, the growth rate forecast for improved trees can be obtained only on good sites. These same sites, incidentally, are capable of supporting high value agricultural crops like cotton and soybeans. So we may find ourselves attempting to market improved seeds that grow well on sites that are no longer available to forestry. It makes little economic sense to invest heavily in producing genetically improved stock for nonexistent acres. We might better prepare for the future by investing more research and development dollars to provide planting stock that grows reasonably well on marginal and even poor sites.

Several years ago, I pointed out that rates of return for cottonwood plantations in the Mississippi Delta offered an excellent investment opportunity relative to other forestry uses. But the investment was poor relative to nonforestry opportunities on those sites; and since then agricultural acreage has expanded and acreage intensively managed for forestry has declined (Sternitzke and Nelson 1970).

Geographic location of the site is another potential problem. The history of tree improvement includes many failures when well-intentioned managers transported seeds or seedlings outside natural geographic ranges. Again, we are confronted with a much-invested, nothing-gained outcome.

Furthermore, we are continually directed to combine our genetically-improved trees with plantation management, including site preparation, planting, and cultural treatment. We are also admonished to select the best available sites if we plan to establish a plantation -- with or without improved trees.

Wood production per acre is highest when we establish plantations of superior trees on good sites and follow proven methods of intensive forest management. The group, consisting of plantations, good sites, and genetically-improved trees, must march together either to financial success or disaster. Designing our tree-improvement programs to be so dependent upon capital intensive management techniques on the most expensive acres may be a mistake.

Costs of establishing and maintaining plantations are escalating so rapidly that companies are beginning to reassess expansion plans. If plantation costs become prohibitive, we may find that our increased production of genetically-improved stock serves a declining market.

Interest rates on funds for long-term investments are rising. Labor costs also have increased dramatically in recent years. In either case, we confront a growing scarcity of productive resources. Existing investment funds, arising from internal or external sources, necessarily dwindle. The net result for forest industry might be continued ideo-

logical support for plantations of improved trees but lack of money to establish and maintain them.

Other economic forces add to the restrictions on investment capital. In the South, trends are towards harvesting smaller and younger hardwood and softwood trees, with expanding efforts to utilize the entire tree. Processors cannot sit by and await emergence of plantations of large, clear, and straight trees. Survival in the intervening years necessitates adaptation to existing wood supplies of younger and smaller trees. Utilization of these trees requires substantial capital investments in equipment for harvesting, hauling, and processing. This investment capital may have to be reallocated from planned expenditures on plantations of improved trees. Furthermore, as processors and consumers adapt to the use of entire trees of smaller size, the available supply of wood will dramatically increase and premiums for quality wood from improved trees will fall. Certainly, such bleak prospects do not arise from genetic improvement, but from the widespread reliance on capital intensive forest management, with which tree improvement is closely aligned.

If plantation management is becoming prohibitively expensive, some evidence should be emerging that points to failure to maintain plantations or shortfalls in planned rates of planting. In North Carolina, a number of Forest Survey plots containing pine, including plantations, were harvested between 1964 and 1974. Fifty-eight percent of these plots are now occupied by hardwoods (Boyce and Biesterfeldt 1977). Harvested pine stands in Georgia and Virginia experienced a similar fate; over half have reverted to hardwoods. Most of these stands are not plantations and most are in the hands of small, nonindustrial owners. But, loss of plantations from industrial lands has been reported and is included in the data.

The Southern Forest Institute reports that over 157 million superior seedlings were planted in 1975-1976 (Box 1976). But these seedlings occupy only about 200,000 acres, or 0.1 percent of the commercial forest land in the South. It is estimated that more than 1 million acres east of the Mississippi River would have to be reforested to pine each year just to maintain current percentages of loblolly and slash. We are not meeting this goal; instead, we are planting only about 800,000 acres per year and only about 25 percent of these acres receive improved planting stock.

Thus, data suggests that many of our pine forests are reverting to hardwoods and our rate of planting is insufficient to maintain existing percentages of pines. The culprits, in my opinion, are escalating costs and growing scarcity of capital. Small private landowners do not have the money or inclination to invest in intensive forest management. A growing percentage of industry representatives are making expensive adaptations in harvesting, hauling, and processing to accomodate available wood supplies. With limited capital, industry is finding that

circumstances often suggest greater profits through adapting to the forest resource rather than investing so heavily to alter it.

This may seem a depressing note to conclude on, but I feel that these are realistic considerations that offer opportunity as well as consternation. I am merely suggesting that risks are inherent in an alliance of so great a proportion of our tree-improvement efforts with the most expensive forest management regimes. We might realize greater long-run benefits by seeking ways to increase or just maintain acceptable growth rates on poorer sites with less intensive management. Insuring natural regeneration of chosen stock, relying very little on fertilizer application, and developing trees that compete efficiently and produce added wood fiber appear to be research goals that would pay off in terms of enhanced wood supplies. I applaud and am awed by progress in genetic improvement, but feel constrained to point out some economic factors that urge us to align our improved trees with less costly management companions.

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THE GENETIC AND ECONOMIC EFFECT OF PRELIMINARY[1] CULLING IN THE SEEDLING ORCHARD

Don E. Riemenschneider[2]

ABSTRACT.--The genetic and economic effects of two stages of truncation selection in a white spruce seedling orchard were investigated by computer simulation. Genetic effects were computed by assuming a bivariate distribution of juvenile and mature traits and volume was used as the selection criterion. Seed production was assumed to rise in a linear fashion to maturity and then remain constant for the life of the orchard. Expected seed production was used to compute the number of acres that could be planted and volume gain over this acreage was estimated using predicted response to selection and white spruce volume tables. The added volume was compared to the cost of the orchard using the cost-price investment criterion. The cost-price of the volume gain was found to vary little over reasonable intensities of preliminary culling and the optimum intensity remained the same regardless of the juvenile-mature correlation.

Many economic analyses of forest tree improvement projects have already been done (Dutrow 1974). To date, however, most of these studies have dealt with clonal seed orchards (Davis 1967, van Buijtenen and Saitta 1972, Porterfield 1974) rather than seedling seed orchards. I began this study to investigate the genetic and economic potential of a white spruce seedling seed orchard by means of computer simulation. The goals were to determine (1) the effect preliminary culling had on genetic gain and economic return, and (2) the optimum level of culling.

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METHODS

A 4.5 acre white spruce (Picea glauca (Moench) Voss) seedling seed orchard in north-central Minnesota was used as the model population. The orchard initially contained 7,200 individuals representing 239 open-pollinated families. Early measurements of height growth and estimates of heritability have been reported (Mohn et al. 1975).

Although combined family and mass selection is likely to be more efficient, the problem was reduced to a manageable level by assuming that there will be two rounds of mass selection in the orchard. Merchantable volume will be the selection criteria. The first round of selection at 15 years after planting will represent juvenile selection or "preliminary culling". The second round of selection will be at 30 years after planting. This final selection will be on the basis of the mature characteristic. Approximately 450 individuals (or 6.25 percent) will remain after all selections have been completed. Gain from parental selection was not considered. A termination date was set at 50 years after planting.

With this culling schedule there will be two productive stages in the life of the orchard. From 15 to 30 years after planting, genetic gain will be the result of the correlated response of the mature trait to preliminary culling based on the juvenile trait. Seed production during this period should be increasing as the trees approach maturity. After final selection at age 30 genetic gain will be based on both rounds of selection. Seed production should reach its maximum and remain constant.

The intensity of preliminary culling will affect response to selection in both stages and total seed production in the first stage. It was necessary to quantify this effect and then determine the intensity that maximized the potential return on the orchard investment.

The genetic effect of preliminary culling were evaluated by assuming that the two selection criteria are bivariate normally distributed. The technique used in this study has been described by Brown (1967) and applied to a tree improvement problem by Namkoong (1970). The genetic effects of selection were computed as follows:

Let: u = the mature trait and

v = the correlated juvenile trait,

Assume that u and v are bivariate normal with phenotypic correlation r_p genotypic correlation coefficient r_a, and heritabilities h^2_u and h^2_v.

If $x = \frac{u - \bar{u}}{\sigma_u}$, $y = \frac{v - \bar{v}}{\sigma_v}$, and σ_u and σ_v are the phenotypic standard deviations of u and v, then x and y are standard bivariate normal with:

$\bar{x} = \bar{y} = 0$ and,

$\sigma_x^2 = \sigma_y^2 = 1.0$.

The selection procedure in terms of the univariate distribution of x and y will be to discard all individuals where y<b at the preliminary culling and to discard all individuals where x<a at the final selection.

To determine the culling levels, univariate y is divided into 40 intervals, and for each level of culling at y - b, the truncation point x = a is obtained, which gives $p\ (a,b: r_p) = P$ where:

$p\ (a,b: r_p)$ = the fraction of the population retained after truncation of a bivariate distribution, discarding all individuals where y<b and x<a, and

P = the portion of the population the breeder wishes to retain after both rounds of selection (6.25 percent).

The gain made in x by truncation selection in y is:

$$\Delta G_{x/y} = i_1\ h_x\ h_y\ r_a \quad \text{(Falconer 1960)}$$

where: i_1 = the selection intensity in y,

$h_x = (h_x^2)^{\frac{1}{2}}$,

$h_y = (h_y^2)^{\frac{1}{2}}$, and

τ_a = the genotypic correlation between x and y.

Assuming a large population, the selection intensity i_1 is computed by the stochastic relation $i_1 = \frac{Z\ (b)}{Q\ (b)}$

where: Z(b) = the ordinate of N (0,1) at y = b, and

Q(b) = the standardized univariate normal density function integrated from b to $+\infty$.

$\Delta G_{x/y}$ represents the genetic superiority of seed produced between the preliminary culling and the final selection.

When the final selection is made by truncation at x = a, the total genetic gain obtained in x is given by:

$\Delta G_x = i_2 h_x^2$ (Falconer 1960) where: i_2 = the selection intensity in x after truncation selection at y = b and x = a, and

h_x^2 = the heritability of x.

The method of computing i_2 is described by Young and Weiler (1961) and a mathematical proof is given by Weiler (1959). The expected mean of x after both rounds of selection ($\bar{x}'$) can be written in terms of a univariate distribution.

$$E(\bar{x}') = \frac{Z(a)\ \ Q(A) + r_p\ Z(b)\ \ Q(B)}{P}$$

where: $A = \frac{b - r_p a}{\sqrt{1 - r^2 p}}$, $B = \frac{a - r_p b}{\sqrt{1 - r^2_p}}$, and

r_p = the phenotypic correlation coefficient.

And because the original distribution of x has mean 0, $\bar{x}' = i_2$.

ΔG_x represents the genetic superiority of seed produced after the final selection.

$\Delta G_{x/y}$ and G_x are expressed as a percent of $\bar{u}$ by multiplying times the coefficient of variation of u.

The amount of seed produced by an orchard is equally as important as genetic gain. Seed production was assumed to begin 15 years after planting and then rise linearly to a constant level of 250 cones/tree/year (Nienstaedt and Jeffers 1970) at 30 years after planting. Total yearly seed production was then computed as the average per tree production times the number of trees in the orchard.

The volume growth in excess of normal yield produced by this improved seed is:

$$V = \frac{A \cdot Vn \cdot G}{100}$$

where: A = the number of acres regenerated based on 8 x 8 foot spacing and 70 percent average seed viability,

Vn = normal yield per acre on medium site land with 40 year rotation (Stiell and Berry 1973), and

ΔG = percent genetic superiority of the seed depending on the stage in which it was harvested.

The schedule of activities that were included as costs are shown below:

Year	Activity
Selection Phase	
1	Select parents and collect open-pollinated seed
1	Extract and prepare seed
Nursery Phase	
2	Prepare bed
2	Sow
2,3	Maintain seedbed
4	Transplant
4,5	Maintain transplant bed
6	Lift and wrap stock
Orchard Phase	
6	Purchase land
6	Prepare site
6	Plant
6-55	Maintain
20	Measure
20	Mark and cull
21-35	Collect seed
35	Measure before final selection
35	Mark
36-55	Collect seed

Estimates of the cost of each activity were obtained from individuals who participated in the project or from organizations that had experience in similar operations. An inflation rate of 4 percent was included when estimating future costs. Costs that occur between 15 and 20 years after planting, especially seed collection, will vary with culling intensity. As culling intensity is increased, these costs are reduced.

The investment criterion used to evaluate culling alternatives was the cost-price relation (Lundgren 1973). This is the price at which a product must be sold in order to break even on the investment. Because output is produced over a period of years, this price was necessarily assumed to be constant. It was computed by dividing cost by output ($/cord) in terms of present equivalents. The present equivalents of cost and output were obtained using standard discounting procedures and an interest rate of 8 percent.

In the Minnesota orchard, heritability for height at 8 years after planting was 0.35 with a coefficient of variation of 34 percent. On the basis of these estimates it was felt that a heritability of 0.30 for volume with a coefficient of variation of 20 percent would provide a conservative estimate of gains from selection.

Seed produced from age 16 to age 30 ($\Delta G_{x/y}$) and from age 31 to age 50 (ΔG_x) were found to be genetically superior (fig. 1 and 2). When levels of culling are low, $\Delta G_{x/y}$ is also small because i_1 is small. But when levels of culling rise, $\Delta G_{x/y}$ also rises appreciably. If all selection is done at age 15, $\Delta G_{x/y}$ is 6 percent, 8 percent, and 11 percent for correlations of 0.5, 0.7, and 0.9, respectively.

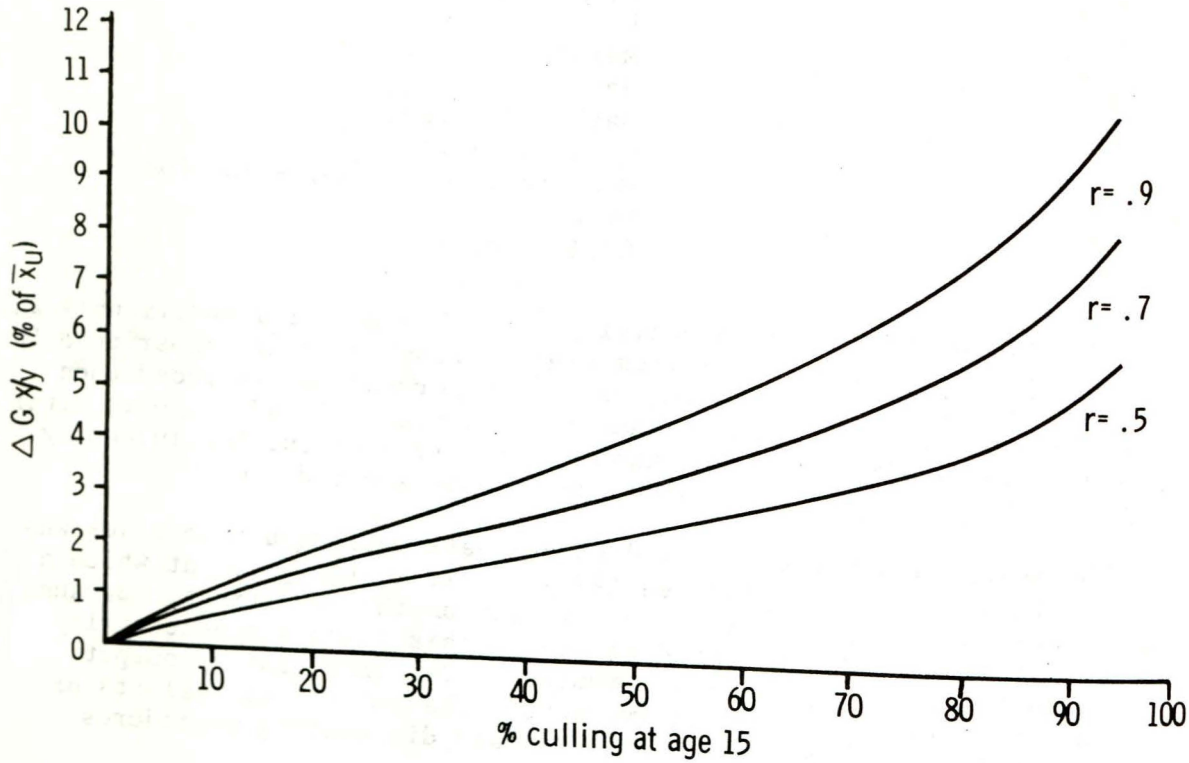

Figure 1.--Gain in the final selection criterion with preliminary culling.

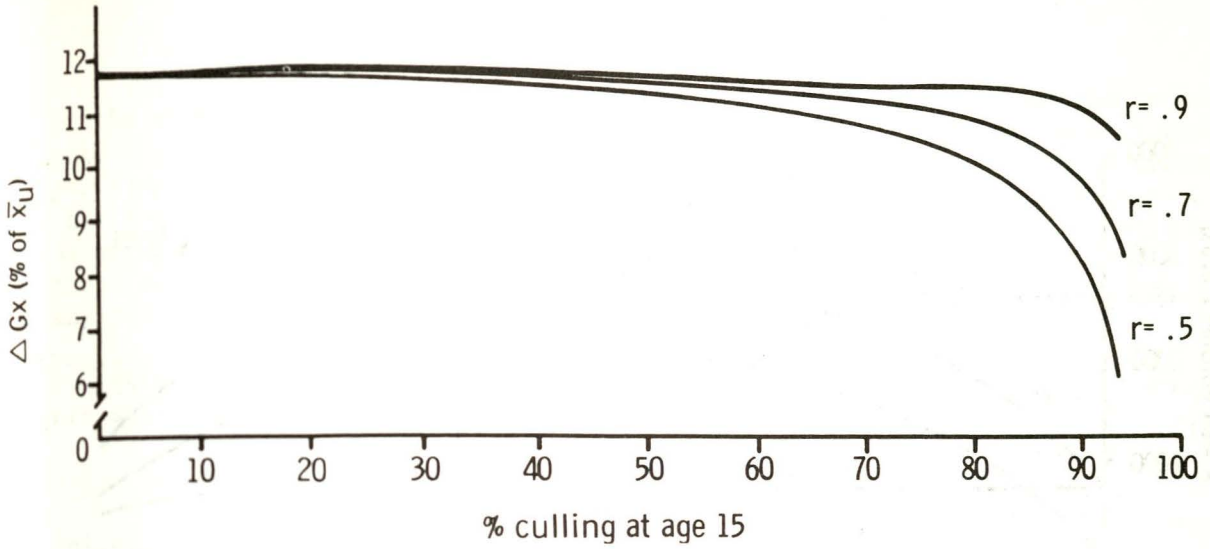

Figure 2.--Gain in the final selection criterion after two rounds of truncation selection.

At low and intermediate levels of culling, ΔG_X does not decrease appreciably. Maximum ΔG_X is 12 percent when $i_1 = 0.0$ for all correlations. For correlations of 0.5, 0.7, and 0.9, ΔG_X is reduced to 6 percent, 8 percent, and 11 percent if all selection is done at age 15. The drop in ΔG_X is not great until high levels of culling are reached and the drop is more severe if the correlation between traits is low. At the maximum culling level $\Delta G_{X/y}$ should equal ΔG_X because there will be no second round selection. The fact that the values obtained agree closely provides a good check on the computational procedures employed.

The present equivalent of the predicted volume in excess of normal yield maximizes at an intermediate point (figs. 3 & 4). This is because th volume gain from pre-final selection seed is a function of both seed production and $\Delta G_{X/y}$, which vary inversely as culling level increases, the curve maximizes at an intermediate point. Because stable seed production is assumed after year 30, only genetic gain after 30 years influences volume gain. The curve decreases slightly at high levels of culling. The importance of early seed production and selection can be seen in the relative heights of the two curves. Although this early production is low, it contributes significantly to the total volume gain when considered for the entire investment period. More volume is produced at higher correlations, but the maximum point is reached at about 50 percent culling.

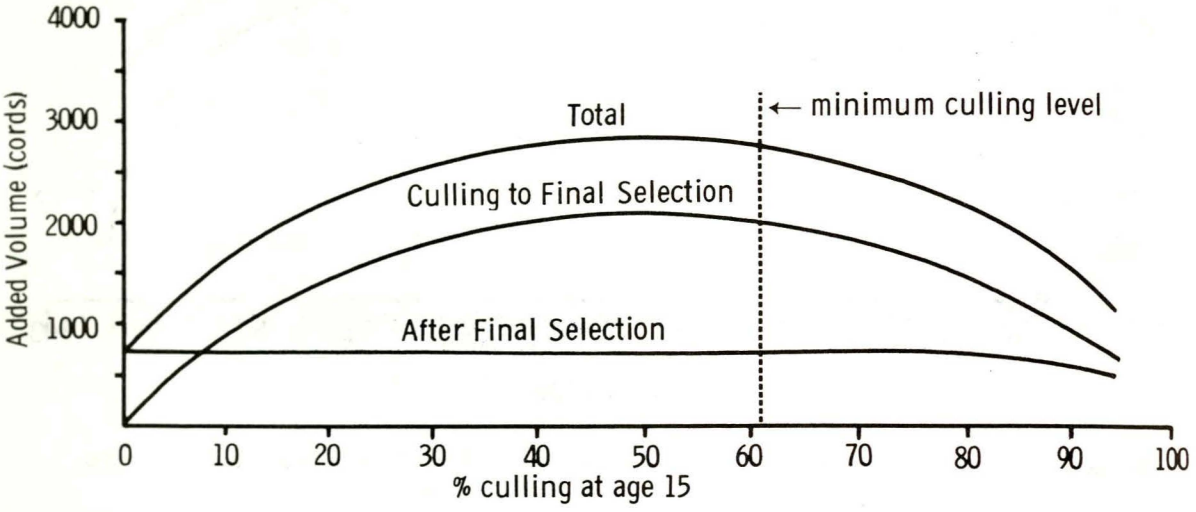

Figure 3.--Gain in volume at rotation age (juvenile-mature correlation = .7) (present equivalent).

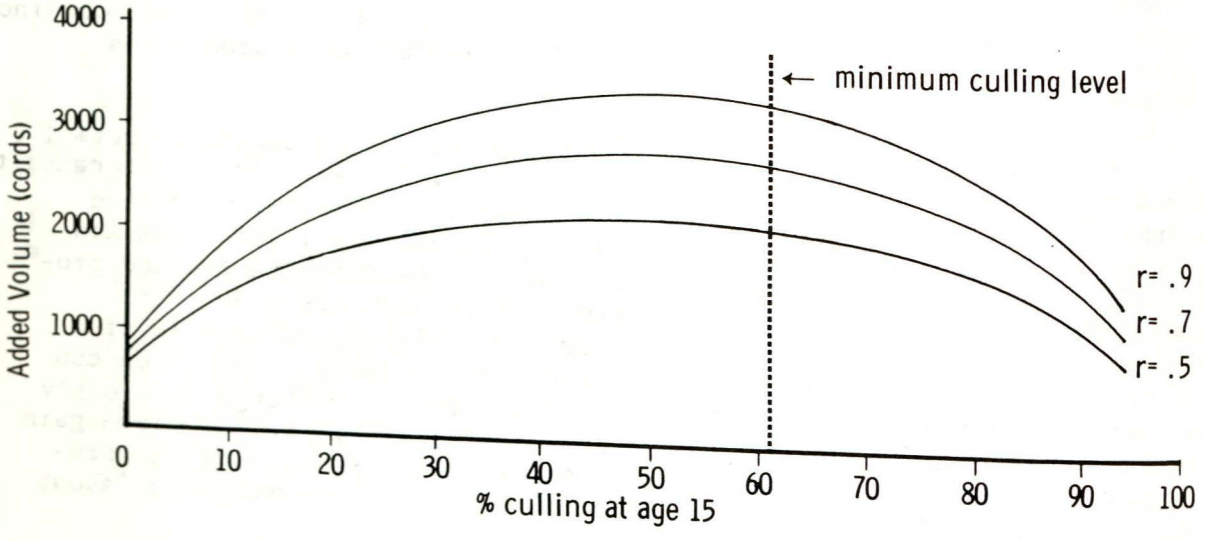

Figure 4.--Total gain in volume at roation age (present equivalent).

Total orchard seed production was assumed to be a linear function of the number of trees in the orchard. Obviously at low culling levels this linear relation will not exist because crowding will reduce average per tree production. Stocking data (Stiell and Berry 1973) indicate that overcrowding and mortality will occur in the model orchard unless 61 percent of the trees are removed at age 15. This would seem to be the minimum intensity of culling above which volume gain predictions may be accurate.

The present value of costs that are not affected by culling level are shown below:

Activity	Cost
Selection and seed preparation	$ 693.33
Nursery handling	660.39
Land purchase and site preparation	131.80
Planting, maintenance, pre-culling measurements	1,952.30
Seed collection after final selection	2,769.28
TOTAL	$6,207.10

The total present cost ranged from $14,282 at the minimum culling intensity of 61 percent to $8,131 at maximum culling.

The cost price of the gain in volume is a u-shaped curve (fig. 5). The cost/cord is high at low culling levels where $\Delta G_{x/y}$ is small; it reaches a minimum at 73 percent culling and increases at high culling levels because seed production is reduced. Again the curves optimize at the same culling level regardless of the correlation between traits. The minimum price does vary with the correlation coefficient.

The shape of the cost-price curve is flat for the most likely culling intensities. This indicates that a good deal of flexibility is permitted before the cost of production is measurably affected. Also, although the juvenile-mature correlation affects the economic feasbility of the program as a whole, it has little impact on the optimum intensity of preliminary culling.

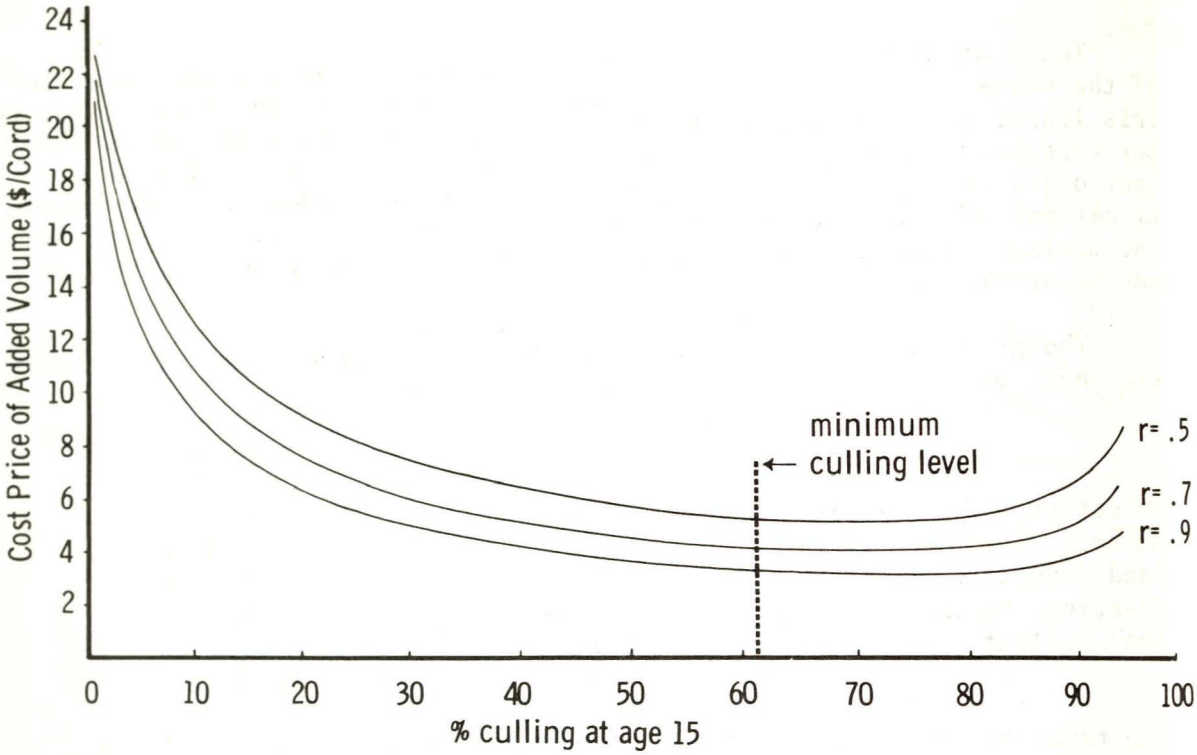

Figure 5.--Cost-price of volume gain.

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DAMAGE TO SOUTHERN MICHIGAN CONIFERS DURING THE WINTER OF 1976-77

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ABSTRACT.--In southern Michigan, the winter of 1976-1977 was marked by unseasonably cold weather in early December, prolonged cold weather in December and January, severe drought at the onset of cold weather, and by higher than average absolute minimum temperatures. Damage, presumably from the early December cold weather, was severe to southern seedlots of ponderosa pine, Austrian pine from the western Mediterranean, Cryptomeria, Spanish origin Scotch pines planted in low-lying areas, and southern seedlots of white fir. Surprisingly, there was little damage to eastern white pine from the southern Appalachians, southwestern white pine from Arizona and New Mexico, baldcypress and Metasequoia. Comparisons between laboratory and field experiments indicate that laboratory experiments can give useful information about the temperatures at which appreciable injury can be expected in the field.

The winter of 1976-77 was the coldest in memory for many parts of the Eastern United States. Southern Michigan was no exception. It seemed desirable to assess the damage caused because there is a good possibility that woody plants which survived that winter will continue to thrive for many years.

HOW SEVERE WAS THE WINTER?

The winter was especially severe as regards average temperature, duration of cold weather, and drought. For Lansing, the average December temperature was 11.5°F below the 40-year average, and December had a

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total of 1,445 degree-days (below 65°F) as compared with normal totals of 1,000-1,100. In Lansing, there was a period of 54 successive days when the temperature did not rise above freezing. Records were also broken in Battle Creek and other southwestern Michigan cities.

On December 3, 1976, there were unseasonably low temperatures. That night the temperature dropped to -7°F at Lansing and Battle Creek and to -15°F at the Kellogg Forest. Absolute minimums later in the winter were not extraordinary, however. At Lansing the lows were -17°F on December 30 and January 9, as compared with a low of -25°F the previous January 18. At Battle Creek the lows were -10° and -9°F, respectively on December 31 and January 28, as compared with a low of -12°F the previous winter.

The winter was also marked by severe drought conditions. Precipitation had been below normal the previous two years. These drought conditions continued into 1977 and by midsummer were so severe that many small ponds disappeared.

WHEN DID THE DAMAGE OCCUR?

Only for ponderosa pine is it possible to estimate the date on which the damage occurred. For other species it is possible only to say that severe damage was evident by the end of February.

In ponderosa pine, one of us (DeHayes) was in the process of making a detailed laboratory study of cold hardiness. This entailed collecting needles and twigs, freezing them at various temperatures in the laboratory, and measuring electrically the resultant cell damage. Needle collections of nonhardy types made shortly after the December 3 cold spell were mottled. Also, laboratory tests made at the same time indicated cell damage similar to that induced by artificial freezing.

NATURE OF THE DAMAGE

Observations on ponderosa pine indicated that the initial injury to a needle could be at any place, from base to tip. If at the tip, only the tip subsequently became brown. If at the base, the entire needle became brown. Generally, there was a strong correlation between the proportion of needles attacked per tree and the amount of damage per needle.

Cambial injury was less common, and was nearly limited to twigs with 100 percent leaf damage. Some pines were completely brown by mid-February but still retained green cambium and produced new growth later in the spring. Mortality was generally limited to trees which had been damaged in previous years or which were in frost pockets subject to extraordinarily low temperatures.

THE MOST SERIOUSLY DAMAGED SPECIES

Ponderosa Pine (Pinus ponderosa)

Damage was most noticeable on the trees of the ponderosa pine range-wide provenance tests established in 1962 at the Kellogg and Russ Forests with O. O. Wells' material. Although these plantations had suffered some damage in previous years, the 1976-77 injury was by far the worst. Damage was nil to the all-interior provenance test established in 1968 with material supplied by the U.S. Forest Service.

In the range-wide tests, trees from California (all parts except the extreme northeast) were hit hardest. Already weakened by cold injury in other years, few seemed likely to survive for more than a year or two. Next worst were trees from Arizona and New Mexico. Most of them seemed destined to survive; their growth rates were already much reduced because of injury in previous years. Trees from the Willamette Valley in western Oregon suffered moderate winter injury (30 to 40 percent of the leaf area brown) in 1976-77 although they had been damaged little in previous years. They had been among the tallest and grew well in 1977 despite their injury.

Ponderosa pines from eastern Oregon, Washington, Idaho and the interior states north of Colorado came through the winter unscathed. According to laboratory tests they had achieved sufficient hardiness even by early December to withstand severe cold.

Another experiment at Kellogg Forest which was a casualty of the cold weather was a plantation of Placerville-produced hybrids which had been planted in the early 1950's. A great number had California ponderosa pine as one parent. Perhaps because they had been planted with better air and soil drainage than found in the provenance test, they had remained thrifty for many years. However, all with California germplasm succumbed after the 1976-77 winter.

In addition to the experimental plantations, Kellogg Forest has four commercial plantations of ponderosa pine which are 35 to 40 years old. They seem to be of Washington or northern interior origin. These were not harmed by the cold although a small percentage of the trees have serious disease problems.

Around the Lansing area and at scattered locations elsewhere, ponderosa pine has been used as an ornamental, and many trees are more than 50 years old. Most of these ornamentals escaped injury.

Austrian Pine (*Pinus nigra*)

Austrian pine has been a favorite for planting along roadsides in southern Michigan because it is resistant to salt. While disease has been a problem on older trees, damage from cold had rarely occurred on trees south of the Bay City-Muskegon line.

Winter injury was very noticeable after the 1976-77 winter, however. In the range-wide provenance test established in 1961 at Kellogg Forest, most trees from Corsica and southern France had 60 to 70 percent of the foliage turn brown. They had been similarly injured in the nursery but had suffered only minor damage in recent years.

Seedlots from Spain, other parts of France, the Greek Islands, and the Greek Peloponnesian Peninsula were damaged on 10 to 30 percent of their foliage. Trees from Austria, Yugoslavia, northern Greece, Turkey, and the Crimean SSR were not hurt.

Many non-experimental roadside trees, most of them thought to be of Austrian origin, were also damaged. It was typical to find heavy injury (50 to 75 percent of the foliage brown) on one or two of a group of 10 trees, with no injury on the remainder. The damaged trees grew normally during 1977.

White Fir (*Abies concolor*)

At both Russ and Kellogg Forests we have provenance tests including seedlots from the Rocky Mountain portion of the range. The Russ plantation, on a low-lying site, was damaged most heavily. In the most sensitive seedlots, 100 percent of the leaf area was injured at Russ as compared with 30 to 40 percent at Kellogg.

There was an interesting contrast in hardiness between two Arizona seedlots originating 1° latitude and 500 ft elevation apart. The most severely damaged seedlot in the experiment was the fastest growing one, No. 2427, from an elevation of 8,100 ft and a latitude of 32° 27'N on Mt. Lemmon in extreme southern Arizona. There was very little damage to moderately fast growing seedlot No. 2423, collected from an elevation of 7,600 ft and a latitude of 33° 20'N. Other seedlots from central Arizona and southcentral New Mexico suffered slight to moderate damage (10 to 30 percent of leaf area injured). Slowly growing trees from farther north suffered no damage.

The California-Oregon provenance test organized by W. J. Libby is also represented by a small plantation at the Kellogg Forest. Practically the entire population from these states exhibits introgression from grand fir (*Abies grandis*), which is a lower elevation and less hardy species of the Pacific Northwest. Trees in this experiment had suffered some winter injury in most previous years, but not to the same extent as in the winter of 1976-77 when they became red-brown.

White fir suffered less permanent damage from the cold than did the pines. The most badly damaged trees from Arizona and New Mexico grew as well in 1977 as in previous years. Even many of the California trees which were red-brown at the end of the winter produced new shoots in the spring.

Eastern White Pine (*Pinus strobus*)

In southern Michigan, eastern white pine from the southern Appalachian Mountains holds special promise. It has grown rapidly for 18 years and is recommended for commercial planting. We were apprehensive as to the effect of the extreme cold weather on the fast growing seedlots. In three widely separated plantations south of Lansing there was no winter injury, even to trees from northern Georgia. We have not had a chance to re-examine a 10-year-old plantation of southern Appalachian trees located in the northern half of the Lower Peninsula.

The long winter was, however, an extraordinarily hard one for eastern white pines located along roads throughout the Lower Peninsula. Apparently most of the damage is due to blowing salt dust, rather than spray. Where there was considerable open space next to the road, as along a thruway, there was serious needle burn on trees 200 to 300 ft to leeward of the road. In the case of roads bordered by trees, damage was usually limited to within 50 ft of the roadway. Whereas in previous years such damage was usually confined to young trees, some 70-80 ft specimens were brown after the 1976-77 winter. Trees away from roads were not affected, even though in the open.

Southwestern White (*Pinus strobiformis*) and Limber Pines (*P. flexilis*)

Southwestern white is a 5-needled pine native to Arizona, New Mexico, and a small part of Texas. It has thick, blue-green foliage, grows at moderate rates, and has been in much demand for Christmas tree planting in recent years. Even the fastest growing seedlots from Texas came through the winter unscathed at both the Russ and Kellogg Forests. Also, older trees in a Battle Creek arboretum were undamaged by the winter.

Limber pine is a slow growing relative from Colorado, Utah, and north. Peculiarly, it suffered apparent winter injury. On a very small number of trees, 30 to 40 percent of the foliage was brown at the end of the winter.

Western White Pine (*Pinus monticola*)

Western white pines of north Idaho origin were planted at Kellogg Forest and at the Tree Research Center on the MSU campus in East Lansing in 1970. They appeared hardy until the winter of 1976-77 when most of the trees on the MSU campus were severely injured (60 to 90 percent of

the needles brown). At Kellogg Forest, damage was severe for trees planted within 10 ft vertical distance of the bottom of a ravine but slight for trees planted on a hilltop or on upper slopes.

Scotch Pine (Pinus sylvestris)

Interracial hybrids of Scotch pine have been planted on hilly ground at Kellogg Forest since 1970. In the plantations there are 50 ft elevational differences within relatively short distances. There are some low spots without air drainage. Within 1 ft vertical distance of these low spots, some trees died. Trees in other parts of the plantations suffered no injury.

Scotch pine of Spanish origin has been recommended for Christmas tree planting in those parts of the state with an average January temperature of 20°F or above, or roughly in the southern third of the state. These recommendations will have to be changed because even in those areas some plantations suffered severe needle injury during the 1976-77 winter. Damage was most serious in low-lying areas. In some cases nearly 100 percent of the needles were brown and it appeared that mortality would result. We have not had a chance to evaluate the long-term effects of the damage in plantations with less severe injury.

Sugi (Cryptomeria japonica)

Cryptomeria or sugi is a Japanese conifer which is more accustomed to a Tennnessee than a Michigan climate within its native range. Nevertheless, we have two specimens which have lived for more than 40 years on the MSU campus. One, situated about 10 ft from a tall building escaped serious injury. On the other, situated in the open, more than half of the leaf area was dead by the spring of 1977.

SOME SURPRISING ESCAPES

In view of the severity of the winter, we were not surprised as much by the presence of brown trees in some species as by their absence in others. Douglas-fir is a case in point. At Russ and Kellogg Forests there are provenance tests covering the entire interior portion of the range and one seedlot from western Oregon. These tests were planted from 1965 to 1968. Although late spring frosts had killed newly expanded shoots in several years, there had been no previous winter injury. Nor was there any winter injury following the 1976-77 winter, even to fast growing seedlots from southern Arizona and western Oregon.

Metasequoia is a fast growing conifer from a remote part of China. Charleston, South Carolina is the American city having the climate most similar to that prevailing in its native valley. Only unbounded optimism would have caused people to plant it as far north as Michigan in the first place. However, there was only a slight amount of twig dieback--no more

than in previous years--on the several trees which have been planted on the MSU campus. This was true in spite of the fact that all had been planted in exposed situations.

Baldcypress (Taxodium distichum) was another surprise because its native range extends only as far north as southern Illinois and southern Indiana. We have three old trees on the MSU campus, none planted in a particularly favorable spot. They were just as healthy after the 1976-77 winter as in any other year.

First-generation hybrids between Austrian and Japanese red pines (Pinus densiflora) have been planted at several places in southern Michigan during the past 10 years. They did not suffer any noticeable winter injury even though needles turned brown on some Austrian pines similar genetically to the parents of the hybrids.

CONCLUSIONS FROM THE WINTER

The unseasonably cold weather in early December presumably was responsible for most injury suffered by nonhardy types of ponderosa and Austrian pines. For those species the hardening pattern was such that trees could not withstand low temperatures so early in the season although they withstood even lower midwinter temperatures in previous years. The hardening pattern is not the same for all species, however. For example, forsythia, quince, and several rhododendrons flowered profusely in 1977 although their flower buds had been killed by January cold a few years earlier. Thus, the winter of 1976-77 was a good one to test hardiness in some species, and not a good one in others.

Waiting for an abnormally cold winter is a slow way to select trees for cold hardiness. For that reason, laboratory experiments have been undertaken to determine artifically the temperatures at which freezing injury occurs. Three such experiments performed on provenance test material growing at the Kellogg Forest near Battle Creek provide data applicable to southern Michigan. They are the eastern white pine experiment by Maronek and Flint (1974), the unpublished study of black cherry by Sung Gak Hong of the University of Minnesota (now of the Republic of Korea) and the PhD. thesis work on ponderosa pine by one of the present authors (DeHayes). In eastern white pine, needles of the least hardy types were injured severely if subjected to laboratory temperatures of -25° to -44° (-31° to -42C) from November to January; field-grown trees did not experience such low temperatures and suffered no injury. In black cherry, laboratory temperatures of -40°F (-40C) in January caused twig damage to the least hardy types; field-grown trees did not experience such temperatures and suffered no damage. In ponderosa pine, laboratory determined damaging temperatures in early December were much higher for California trees, slightly higher for Arizona-New Mexico trees, and

slightly lower for Washington trees than the actual low temperature of -15°F (-26C) recorded at Kellogg Forest on December 3. Damage was great for California trees, moderate for Arizona-New Mexico trees, and nil for Washington trees.

Thus, it appears that the laboratory tests of hardiness provide useful information about the temperatures at which appreciable field damage can be expected.

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PERFORMANCE OF NORTHERN WHITE-CEDAR IN CENTRAL ILLINOIS

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ABSTRACT.--Growth and survival of 20 provenances of northern white-cedar in the nursery and to age 12 in 3 test plantings in east central Illinois were examined. Survival at age 12 exceeded 95 percent in all tests. Total height differed significantly among provenances at age 4 in the nursery and at greater ages in all tests except after the first and second growing seasons following extensive and severe foliage injury during the winter of 1970-71. Height rankings of provenances varied between test sites and with age on the same site. Provenances from isolated occurrences south of the main range of the species were shortest at age 12. Lack of a well defined geographic pattern of variation may reflect the influence of localized lowland and upland ecotypes. The excellent performance of the species in all tests supports its use for a variety of types of barrier and shelter plantings.

Northern white-cedar (Thuja occidentalis L.) is widely distributed on a variety of lowland and upland sites in southern Canada from Manitoba to Nova Scotia and in the United States and northeastern Minnesota, northern and eastern Wisconsin, northern and central Michigan, New York, and northern New England. It occurs locally in Illinois, Indiana, Ohio, southern New England, and in the Appalachian Mountains from western Pennsylvania to eastern Tennessee and western North Carolina. Its occurrence in Illinois is limited chiefly to bluffs and cliffs of St. Peter sandstone in extreme northeastern portions of the State and occasionally to rock outcroppings along the Illinois River in LaSalle County. From an 1853 record, it is known to have occurred as far south as Peoria (Jones 1963).

The first range-wide provenance study of northern white-cedar was initiated by Scott S. Pauley in 1964 when he obtained seed collections from 32 stands located throughout the botanical range of the species.

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Seed of various provenances was sown in nurseries in Illinois, Michigan, Minnesota, and Wisconsin for the production of planting stock for tests in these States under the North Central Regional Project NC-51 (currently NC-99).

Early results from these studies suggested to Wright (1976) that northern white-cedar was genetically uniform throughout its range. In contrast, Jeffers (1976) found significant height differences among provenances in a northern Wisconsin and a western Upper Michigan planting 9 years old. He concluded that genetic variation does exist in the species, at least in terms of height growth. On the basis of his finding of no significant differences in the nursery and those of Wright, he speculated that genetic differences may not be expressed when trees are grown under optimum conditions.

This paper reports on the performance of 20 provenances through age 12 in Illinois.

MATERIALS AND METHODS

Three field tests were established in east central Illinois with planting stock raised in the Mason State Tree Nursery, Havana, Illinois. Twenty seed lots were stratified in moist sand at 36-40°F for 40 days prior to being sown on April 11, 1965. The seedbeds were mulched with 1 to 2 inches of well rotted sawdust and maintained as a regular production bed. Germination and yield of 2-0 seedlings varied greatly among provenances; however, these differences were not measured because the quantity and undoubtedly the quality of seed sown varied among provenances. The seedlings were lifted and transplanted into regular nursery beds in a randomized complete block design with three replications on May 17-18, 1967. Total heights of 10 transplants in each plot were measured to the nearest .05 feet before lifting in April 1969.

A 3-row windbreak was established with 2-2 bare root transplants on Drummer silty clay loam (Typic Haploquoll) in the Illini Forest Plantations, Urbana, in April 1969. Trees were planted 6 feet apart in rows spaced 9 feet apart. Each of the 20 provenances was represented by a 6-tree plot in two rows oriented diagonally across the break in each of four replicates. The remaining nursery stock was planted in a nearby holding plot.

A second test, a 3-row audio-visual screen, was established in April 1970 with 2-2-1 stock with intact soil balls, on Sidell silt loam (Typic Argiudoll) and Flanagan silt loam (Typic Aquiargiudoll) on the University Veterinary Research Farm located one-third mile east of the first test. Trees were planted 10 feet apart in the two outer rows and 5 feet apart in the middle row. Rows were spaced 8

feet apart. Each provenance was represented by a 4-tree plot--a row of three trees oriented diagonally across the screen plus an additional tree in the center row at 5 feet spacing. The provenances occurred in up to six replicates if stock was available. Data for only the 17 sources that occurred in the first five replicates (the sixth replicate was destroyed by herbicidal drift from an adjacent field) are reported herein.

The stock remaining in the holding bed was planted as 2-2-2 transplants in April 1971 in a visual screen at the Vermilion River Observatory located approximately 30 miles east of Urbana. The soil, a Vance Silt Loam (Typic Hapludalf), is a timber soil in contrast to the prairie soils of the other tests. Trees were planted with intact soil balls around their roots. Various numbers of trees of 16 provenances, totaling 264, were planted completely at random at a 7-foot spacing in an outer row of a multi-row planting.

Weeds were controlled by mulching or disking the first growing season and subsequently by one or two mowings annually.

Total heights of surviving trees were measured annually except after the first growing season in the Veterinary Research Farm Test, and at ages 9 and 11 in all field tests.

RESULTS AND DISCUSSION

Survival

Tree survival at 12 years of age averaged 99, 98, and 96 percent in the Illini Forest, Veterinary Research Farm and Vermilion River Observatory plantings, respectively. All the mortality was attributed either to pilferage or mowing accidents, so it can be concluded that all provenances survived equally well. Sporadic bagworm infestations have been sufficiently severe on occasional trees to suggest that bagworms might cause lasting crown injury or death. All trees survived the record drought of 1976 and 1977 without apparent injury.

Winter Injury

The foliage of northern white-cedar is subject to winter injury in Illinois. The most severe injury observed in the test plantings occurred during the winter of 1970-71 when most trees in the Illini Forest Plantation incurred some degree of injury and 30 percent lost at least half their foliage. Severity of injury showed no definite geographic pattern. The most severely injured provenances were southern Ontario (UMN 33), northeastern Vermont (UMN 17), and Illinois (UMN 34). The least severely injured provenances were central Minnesota (UMN 18), northern Ontario (UMN 40), and western Upper Michigan (UMN 32). Winter injury was much less extensive on the better drained, more exposed site on the Veterinary Research Farm. In subsequent years,

only minor, isolated instances of winter injury have been noted except during the winter of 1976-77 when most trees in the Vermilion River Observatory test suffered light to moderate injury. The buds were not injured, so all trees recovered.

Total Height

Total height growth in the test plantings has equaled or exceeded that of red, white, and Scots pine on adjacent sites at comparable ages. Contrary to earlier results from tests in the Lake States, significant differences were found at 4 years of age in the nursery and at greater ages in field tests. These significant differences occurred under near optimum growing conditions, but they were obscured after the first and second growing seasons following the winter of 1970-71 when winter injury was unusually severe (table 1).

Table 1.--Significance of differences in total height among provenances

Plantation	Age (years)						
	4	5	6	7	8	10	12
Nursery	**	-	-	-	-	-	-
69-2	-	**	**	NS	NS	*	**
70-4	-	-	-	NS	*	**	**
71-1	-	-	-	-	**	**	**

* Significant at .05 level of probability.
** Significant at .01 level of probability.

Although significant differences were found, ranking of provenance means varied from test to test. Simple correlation coefficients, computed from provenance means at age 4 in the nursery, age 2 in three central Illinois tests and age 9 in a Wisconsin and a Michigan test (Jeffers 1976), are low to moderatly high (.19 - .77) (table 2).

Ranking also varied with age in the same plantation. Changes in rank reflect varying seasonal growth effected by varying environmental factors favoring or disfavoring certain genotypes. A season's growth is a major component of total height at young ages and may be large relative to real differences among provenances, so early results from provenance tests may be less reliable than they are often purported to be. This is illustrated by the change in the relative ranking of two Ontario sources (fig. 1). Trees from northern Ontario source

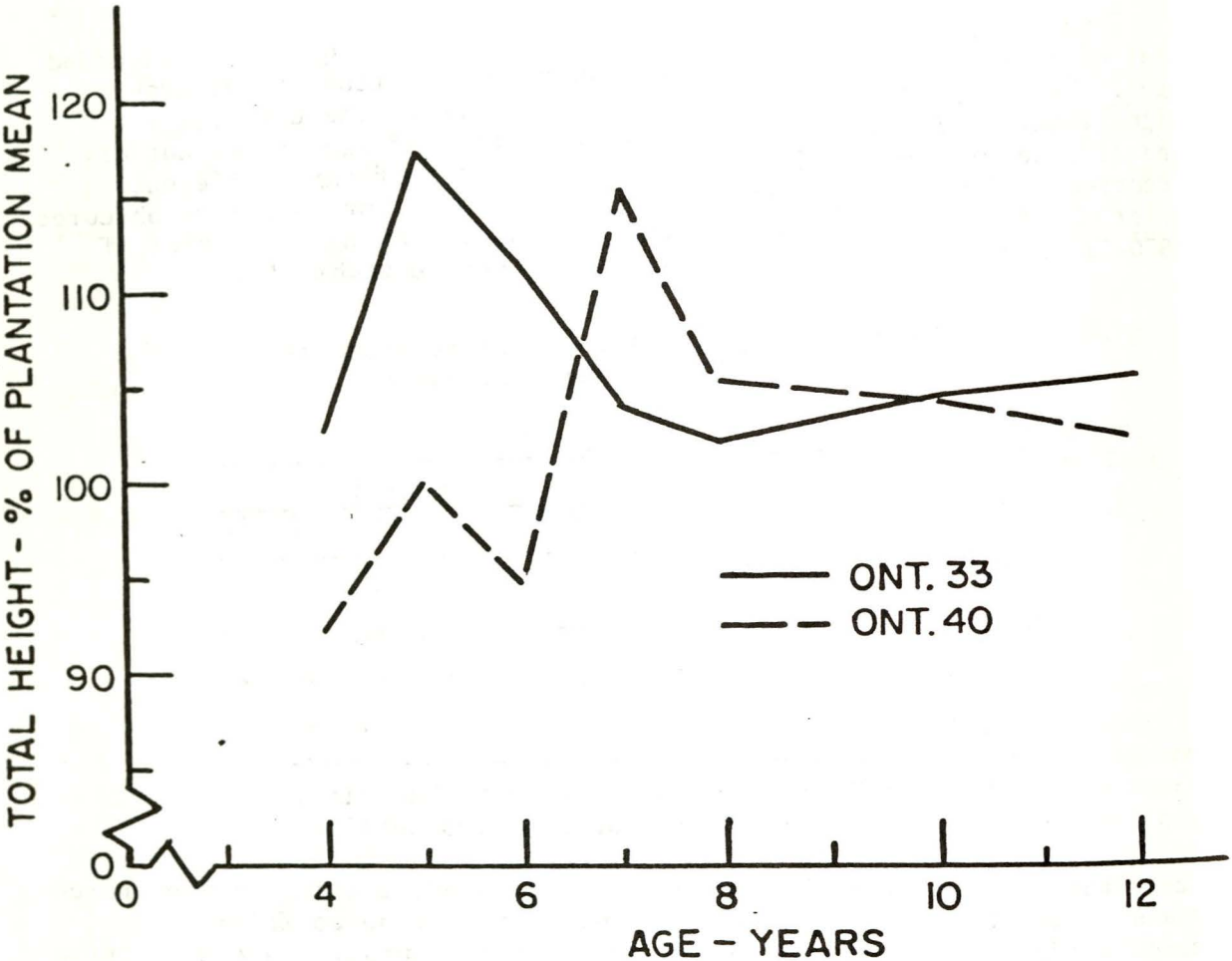

Figure 1.--Relative ranking of provenances with the least (Ont. 40) and the most (Ont. 33) foliage during the winter following the sixth growing season (Illini Forest Plantations).

(UMN 40), which incurred the least foliage injury in the Illini Forest Plantation during the winter of 1970-71 and had previously been one of the slowest growing, was the second tallest after the 1971 growing season. Trees from the southern Ontario source (UMN 33) were the most severely injured.

Table 2.--Between plantation correlations in mean height of provenances expressed as simple correlation coefficients with significance levels

	Nursery (age 4)	69-2	70-4	71-1	Wisconsin
Illinois (age 12)					
69-2	.77*				
70-4	.22	.57**			
71-1	.68**	.73**	.62**		
Wisconsin (age 9)	.20	.40*	.19	.20	
Michigan (age 9)	.26	.49*	.21	.48*	.55**

* Significant at .05 level of probability.
** Significant at .01 level of probability.

Provenance means at age 4 in the nursery and at age 12 in the three field tests, expressed as a percentage of the plantation mean, are given in table 3. Provenances from Washburn County, Wisconsin (UMN 29), Grand Isle County, Vermont (UMN 28), Shawano County, Wisconsin (UMN 21), and Chippewa County, Michigan (UMN 31) were generally the tallest. The first two were among the fastest growing and the last two were average in tests in northern Wisconsin and western Upper Michigan (Jeffers 1976). The slowest growing provenances were from Kenora District, Ontario (UMN 35), Bland County, Virginia (UMN 19), Kane County, Illinois (UMN 34), and Annapolis County, Nova Scotia (UMN 38). The lack of a definite geographic pattern of variation in growth rate and susceptibility to winter foliage injury may reflect the existence of localized lowland and upland ecotypes described by Habeck (1958) and Musselman, Lester, and Adams (1975).

The excellent survival and growth of northern white-cedar in the test planting encourage greater use of this species in Illinois. The dense evergreen foliage and compact crowns create tidy, attractive, living barriers. It would be particularly useful for audio or visual screens, windbreaks, and wildlife cover plantings where space is at a premium.

Table 3.--Relative height of provenances of northern white-cedar in the nursery at 4 years and in three Illinois plantations at 12 years of age

UMN source no.	Seed origin: County	State or Province	Lat.	Long.	Plantation: Nursery	69-2	70-4	71-1
			(deg.N)	(deg.W)	(% of plantation mean)			
19	Bland	VA	37.1	81.1	83	92	93	82
34	Kane	IL	42	88.2	97	93	99	--
37	Herkimer	NY	42.9	75.1	118	105	96	--
33	Blandford Twp.	ONT.	43.3	81	112	105	101	--
36	Clinton	NY	44.6	73.7	95	98	107	97
28	Grand Isle	VT	44.6	73.3	113	103	105	111
21	Shawano	WI	44.6	88.4	105	103	106	115
17	Orleans	VT	44.7	72.0	100	98	96	97[1/]
38	Annapolis	N.SCOT.	44.9	65.2	85	90	94	93
30	Somerset	ME	45.6	70.3	106	99	97	95
22	Forest	WI	45.8	88.9	92	102	103	98
29	Washburn	WI	46	92	107	105	111	108
23	Forest	WI	46.0	88.9	98	99	97	97
31	Chippewa	MI	46.3	84.2	105	104	107	108[1/]
18	Itasca	MN	47	93	103	99	--	--
32	Houghton	MI	47.1	88.6	104	100	97	92
16	St. Louis	MN	48.0	91.6	103	96	--	--
39	Frost Twp.	ONT.	49.5	84.7	108	103	97	111
40	Gurney	ONT.	49.5	82.2	92	102	107	98[1/]
35	Kenora Dist.	ONT.	51.5	90.3	80	94	--	--
Mean (meters)					.40	3.02	3.02	1.86
Probability of >F					<.005	<.01	<.001	<.005

1/ Based on less than 15 observations.

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GEOGRAPHIC VARIATION OF JACK PINE (*PINUS BANKSIANA* LAMB.)[1]

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ABSTRACT.--Ten traits were measured on 10-year-old jack pine grown at Cloquet, Minnesota, from seed collected from 90 provenances. The traits were examined by using analysis of variance and computing correlations for all combinations of 9 traits plus latitude, longitude, and elevation of the seed sources and cluster analyses using the D^2 values from the Mahalanobis distance function. Results of the ANOVA showed that seed sources differed greatly for the 10 traits measured and results of the cluster analysis showed that populations can be geographically defined.

Jack pine (*Pinus banksiana* Lamb.) is an important conifer in North America. It is abundant, grows well on drier, sandy soils, has rapid early growth, and is suitable for pulp and solid wood products. Its range extends from northern New England and the Maritime Provinces west to the Lake States, Alberta, and the Mackenzie District of Northwest Territories.

Because of its importance in Minnesota, a provenance study was begun in 1940. Geographic variation among provenances in this test was reported by Schantz-Hansen and Jensen (1952, 1954) and Schoenike *et al.* (1959). An additional test was begun in 1951 by P. O. Rudolf and several reports on this experiment have been published (Stoeckeler and Rudolf 1956, Conover 1957, Arend *et al.* 1961, Batzer 1961, Rudolph 1964, King 1965, King and Nienstaedt 1965, and Alm and Jensen 1969. Schoenike (1962, 1976) did a detailed study of jack pine variation in natural stands and reported large amounts of variation in many traits; some characteristics showed variation patterns related to geographic location. A range-wide provenance study was undertaken by Holst and Yeatman (1961) and Yeatman (1964, 1966). Yeatman (1966) found an overall clinal pattern

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of genetic variation resulting from environmental adaptation. The material from the Holst-Yeatman study was widely distributed within the species range; a test in Minnesota is the basis for this report. It is the most recent and comprehensive jack pine provenance test in Minnesota and was planted in 1966. The objectives of study were to (1) examine the variation among seed sources for a number of morphological traits and (2) examine the pattern of this variation in relation to geographic distribution using cluster analysis.

MATERIALS AND METHODS

In May of 1966, 1-1 stock from 90 seed sources were planted at Cloquet, Minnesota (fig. 1). A randomized complete block planting design was used with 4 tree-row plots and 5 replications.

When the trees were 10 years old from seed, the following measurements were taken: (1) total tree height, (2) tree diameter at 1 foot above the ground, (3) bark thickness at 1 foot above the ground, (4) crown width, (5) average number of live branches per whorl, (6) average branch angle, (7) average cone angle, (8) cone serotiny, (9) cone abundance, and (10) type of cone curvature. Measurements were made on the first two living trees in each plot.[3]

Standard Analysis of Variance was used to evaluate all of the traits measured except type of cone curvature. (The nature of the cone curvature data made it unsuited for analysis of variance.) Missing data were handled using the procedures given by Snedecor and Cochran (1972) because less than 2 percent of observations were missing.

Differences due to geographic variation were estimated for the nine traits from the ANOVA table by:

$$\frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2}$$

where σ_G^2 = component of geographic variance,

σ_E^2 = component of environmental variance.

Simple correlation coefficients were computed for every possible pair of 12 variables: 9 traits (as previously listed excluding the type

[3]Details on measurement techniques can be found in "Geographic variation of jack pine (Pinus banksiana Lamb.)" by J. O. Hyun (1976) on file at the Library, College of Forestry, University of Minnesota.

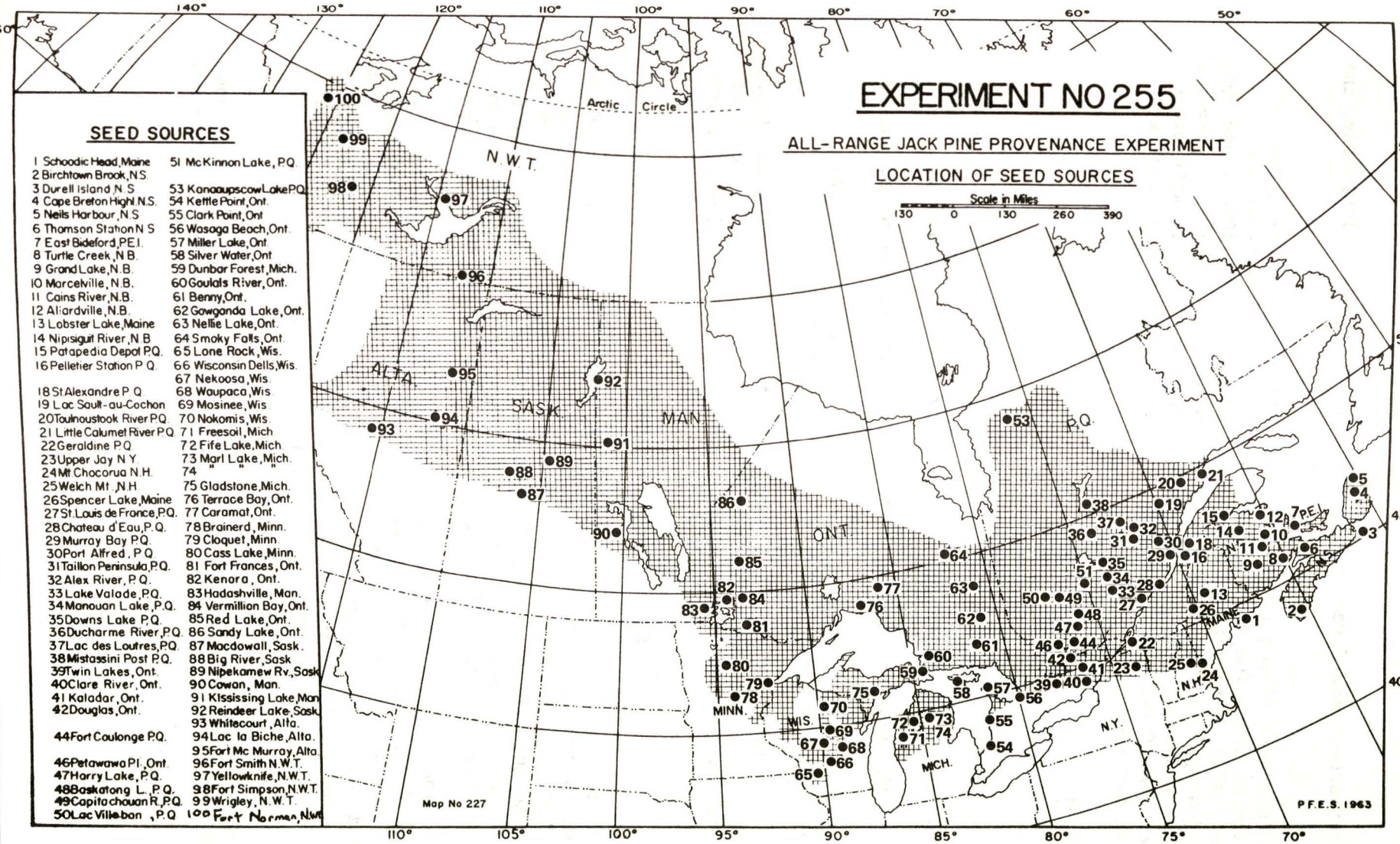

Fig. 1. Natural distribution of jack pine (shaded) and seed sources included in this study (numbered dots). This map was modified from the Map No. 227 for Experiment No. 255 of the Petawawa Forest Experiment Station.

of cone curvature) and 3 geographic gradients (latitude, longitude, and elevation).

The data were fitted to the multivariate analysis using the generalized distance function of Mahalanobis (1936) as treated by Schoenike (1976), Rose (1974), Squillace (1966), Wells (1964), and Wright and Bull (1963). The multivariate analysis made it possible to treat all the traits simultaneously and to compare each seed source with every other one. The formula for generalized distance when more than two traits are compared is:

$$D^2 = \Sigma_i \Sigma_j \; S_{ij}^{-1} d_i d_j$$

where

D^2 = generalized distance,

d_i = the mean population distance for the ith variable,

d_j = the mean population distance for the jth variable, and

S_{ij}^{-1} = the element in the inverse of the covariance matrix corresponding to the ith and jth variable.

D^2 values were computed for the 10 traits measured from the trees from the 90 seed sources[3]. The D^2 values were then used in a cluster analysis to develop numerical values expressing the relative similarities among seed sources.

RESULTS AND DISCUSSION

Significant differences (5 percent or 1 percent level) were found among seed sources for all traits except number of live branches per whorl (table 1). The geographic location of the seed source had the most effect on height, diameter, bark thickness, and crown width; had a moderate effect on cone angle, cone abundance, cone serotiny, and branch angle; and had the least effect on number of live branches per whorl.

King (1965) showed that the seed sources best suited for the Lake States are found within the region. However, results from our study show that height growth of three seed sources from Ontario (42, 55, and 60), three seed sources from Quebec (33, 47, and 48), and one from Manitoba (83) exceeded the local source (79). Therefore, in jack pine breeding, seed sources from other regions can be included in addition to local sources.

Height, diameter, bark thickness, and crown width are highly and positively correlated to each other (table 2). Furthermore, these four

traits show moderate negative correlation with latitude. Only diameter and crown width show significant (1 percent level) negative correlation with longitude. No traits have a significant correlation with elevation. The number of live branches per whorl show a small but significant negative correlation with longitude. A significant, negative, correlation was determined between cone serotiny and cone abundance and a significant, positive correlation between serotiny and longitude. Schoenike (1976) found a significant, positive, correlation between serotiny and latitude, but I could not demonstrate this relation.

Table 1.--Summary of field measurements and analysis of variance

Traits	Plantation mean	Standard deviation	Range of seed source means	F ratio seed source/error	Proportion of geographic variation
					percent
Height	12.8 ft.	1.55 (12)[1/]	6.3-16.0	7.05**[2/]	55[3/]
Diameter	3.2 in.	.41 (13)	1.4- 4.2	6.34**	52
Bark thickness	21.5 $\frac{1}{10}$ in.	3.12 (15)	8.3-33.5	8.54**	60
Crown width	9.7 ft.	1.27 (13)	3.9-12.2	5.74**	49
Branch angle	61.0°	6.42 (11)	46.1-72.6	2.28**	30
No.of branches	4.4	.57 (13)	3.9- 5.2	1.22	7
Cone angle	32.9°	18.84 (57)	3.1-64.8	2.68**	36
Cone serotiny	0.546	.344(63)	.083-1.00	1.82**	21
Cone abundance	12.58	8.24 (66)	1.3-29.2	2.51**	34

1/ Numbers in parentheses are percent of the plantation mean

2/ ** indicates significant at 1 percent level

3/ Proportion = $\frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2}$

As latitude increases, tree growth rate decreases. Also, diameter and crown width decrease as both latitude and longitude increase and are smallest in the northwestern part of the species range.

The D^2 values from the Mahalanobis distance function generated by the computer were used to group the 90 seed sources into clusters.

Table 2.--Simple correlation coefficients (r) for 9 traits and 3 geographic gradients

	1 Height	2 Diameter	3 Bark thickness	4 Crown width	5 Branch angle	6 No. of branches	7 Cone angle	8 Cone serotiny	9 Cone abundance	10 Latitude	11 Longitude	12 Elevation
1. Height	1.0				1/							
2. Diameter	.887**2/	1.0										
3. Bark thickness	.703**	.806**	1.0									
4. Crown width	.783**	.839**	.673**	1.0								
5. Branch angle	-.038	-.062	-.138	.064	1.0							
6. No. of branches	.360**	.337**	.146**	.261**	.099*	1.0						
7. Cone angle	-.091*	-.049	.095*	-.064	-.069	.032	1.0					
8. Cone serotiny	.025	-.010	-.030	.002	-.053	.009	-.028	1.0				
9. Cone abundance	.297**	.305**	.090*	.353**	.091*	.198**	-.253**	-.101*	1.0			
10. Latitude	-.393**	-.409**	-.453**	-.453**	.065	-.063	-.147**	.076	-.096*	1.0		
11. Longitude	-.073	-.151**	-.037	-.220**	-.125**	-.090*	-.138**	.124**	-.134**	--	1.0	
12. Elevation	.013	-.025	.009	-.067	.009	-.051	-.077	.042	-.021	--	--	1.0

1/ Coefficients in the block are based on data of 5 replications and others are based on 3 replications.

2/ Significant at 1 percent level (**) or 5 percent level (*).

Then, I plotted a dendrograph on the computed within- and between-cluster distances. On the dendrograph, degree of similarity was expressed by the distance between clusters--the shorter the distance between clusters, the more similar they were to each other.

Examination of distances between seed sources and distances within prospective clusters led to the decision to divide seed sources into five final clusters. Cluster A contains 30 seed sources mostly from Ontario, southern Quebec, and eastern Manitoba (table 3 and fig. 2). In cluster B there are 17 seed sources mostly from eastern Quebec, southern Ontario, and along the St. Lawrence River plus several from Alberta, Manitoba, and western Ontario. Three seed sources from the Northwest Territories and Alberta in the western portion of the natural distribution of jack pine plus one source from northwestern Quebec make up cluster C. Cluster D contains 25 seed sources mainly from the eastern portion of the species range (southern Ontario, southeastern Quebec, northern New York, New Hampshire, Maine and Nova Scotia. The last and most distinct cluster, E, is made up of sources primarily from the Lake States. They are taller, have broader crowns, more accute branch angles, larger cone angles, higher serotiny frequency, and fewer cones compared to plantation averages.

Table 3.--Seed sources after grouped into clusters

Clusters	No. of seed sources	Seed sources
A	30	51, 63, 48, 33, 83, 47, 49, 60, 26, 76, 89, 64, 50, 57, 44, 82, 86, 58, 46, 40, 8, 42, 84, 10, 87, 55, 90, 91, 85, 81
B	17	31, 41, 16, 39, 88, 30, 32, 62, 15, 18, 4, 13, 95, 77, 92, 94, 20
C	4	53, 97, 93, 99
D	25	27, 35, 98, 12, 38, 19, 25, 36, 5, 28, 34, 29, 11, 70, 6, 9, 56, 7, 23, 21, 61, 22, 24, 37, 14
E	14	68, 72, 69, 66, 65, 78, 67, 80, 73, 79, 71, 74, 75, 54

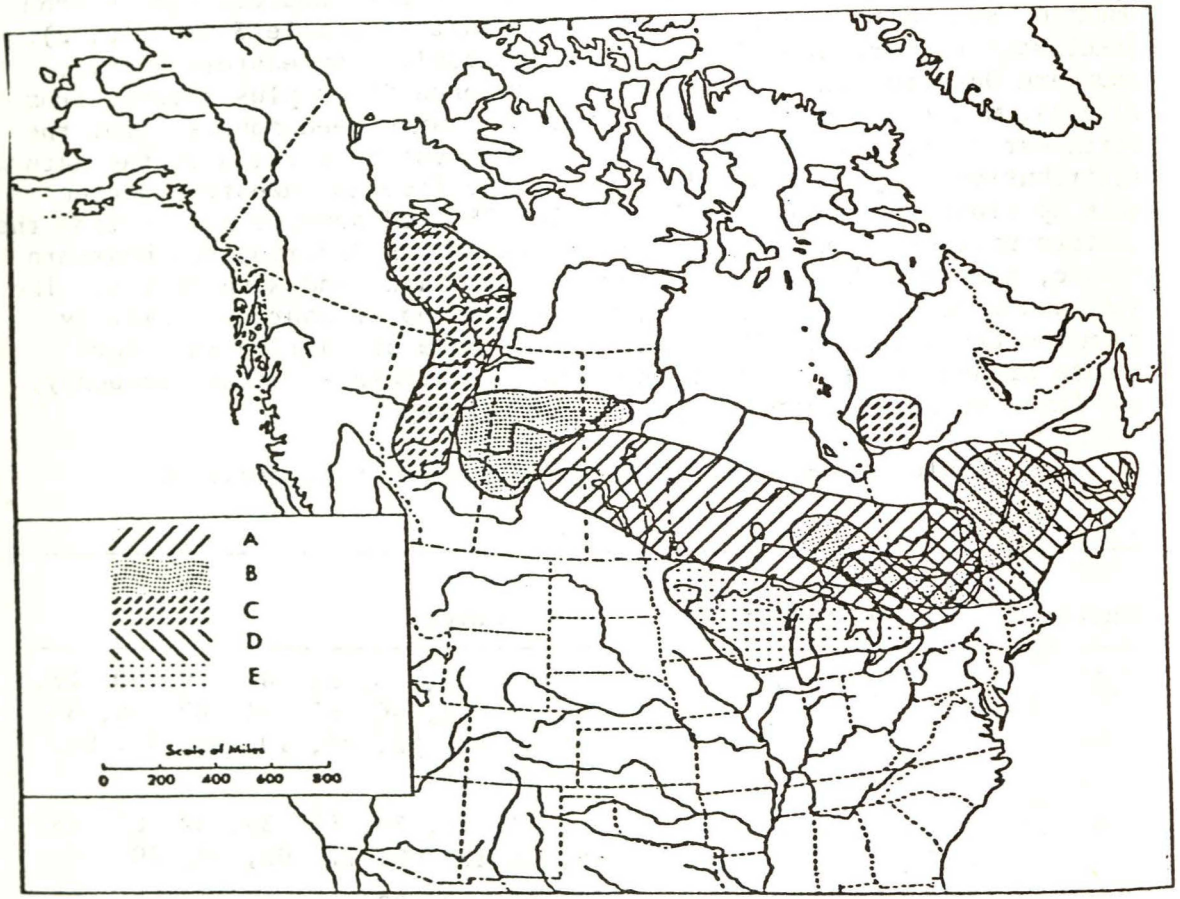

Fig. 2. Proposed subpopulations derived from the dendrograph of the cluster analysis.

Clusters C and E are geographically the most distinct but cluster A is also fairly clear (fig. 2). Clusters B and D do not occupy clearly separated geographic regions.

These results were compared with the results of the study of geographic variation in jack pine done in natural stands by Schoenike (1976). Cluster E, the Lake States group, covers an area similar to that occupied by two of his groups. However, clusters A, B, C, and D are different from any of Schoenike's groups. This is not unexpected because different materials were used for the two studies, the data were collected in different ways, and different traits were evaluated. However, both studies suggest somewhat similar geographic grouping that can be used in working with the species.

CONCLUSIONS

1. There are large variations among seed sources in the 10 traits measured.

2. Most traits that are related to tree growth show large differences in seed source location.

3. Latitude of seed source affected height, diameter, bark thickness, and crown width.

4. Cluster analysis provided a strong indication of geographically defined populations that can be used for tree and seed source improvement.

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INTRASPECIFIC VARIATION IN HIMALAYAN WHITE PINE, PINUS GRIFFITHII [1]

John B. Genys[2]

ABSTRACT.--Twenty-one seed sources of Himalayan white pine (Pinus griffithii McClel.) (11 from native stands and 10 from planted trees) were studied in Maryland's State Forest Tree Nursery and in 11 plantations in Maine, Maryland, Michigan, Illinois and North Carolina. In the nursery, intraspecific variations were observed in leaf lengths, time of bud-set, tendency for secondary growth, and 2-year heights and diameters. Survival rates in Maryland's Piedmont Plateau and coastal plain, and in North Carolina were satisfactory. In Maine, New York, Michigan, Illinois and Maryland's Allegheny Mountains, P. griffithii either completely failed or showed very poor survival. Some strains from Pakistan were more winter-hardy than those from India or Bhutan. In two Maryland plantations best growth rates were attained by populations from Uttar Pradish in India, Shorgan in Pakistan, and from three locations of planted trees. In New York and North Carolina plantations, Himalayan white pine was attacked by white pine weevil (Pissodes strobi Peck).

Himalayan white pine (Pinus griffithii McClelland) was named and described in 1854. Some synonyms are P. excelsa Wallich ex. D. Don, P. wallichiana A. B. Jackson, and P. nepalensis De Chambey. In India, common names are blue pine, kail or biar. Some writers have called it Bhutan pine, Bohtan pine, Nepal pine, or because of white granules on its leaves -- "pine of tears" (Tränenkiefer).

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[1]Contribution No. 774 of the Center for Environmental and Estuarine Studies; and Contribution No. 5351 of the Maryland Agricultural Experiment Station.

[2]The author is Professor and Chairman at Inland Research Laboratory of CEES, University of Maryland, College Park, Maryland.

Morphologically, Himalayan white pine is a close relative to other white pines of subsection Strobi (Konar 1960). In comparison to eastern white pine (P. strobus L.), this species has longer, drooping, more bluish green leaves, larger cones (15 to 30 cm long), and larger seeds. In its native range, it reaches heights of 100 to over 160 feet. P. griffithii includes several known horticultural forms: P.g. form monophylla Carr. (needles in a single bundle), P.g. form zebrina Croux (needles golden-yellowish with white cross-stripes), and P. g. form nana R. Smith (dwarf-like). However, there are no described botanical varieties.

The natural range of Himalayan white pine extends along the southern slopes of the western and central Himalayan Mountains from eastern Afghanistan, through northern West Pakistan, southern Kashmir, Jammu and Punjab provinces of India, Nepal, Sikkim and Bhutan, to isolated populations in Assam, eastern Tibet and northern Burma. It is most common at elevations from 6,000 to 10,000 feet, but occurs as low as 4,000 feet and as high as 12,500 feet. Within this range, P. griffithii is the only pine of subsection Strobi; therefore, it does not cross naturally with other pines in its native habitats. It has been crossed artifically with other white pines, and the hybrid of P. griffithii x P. strobus grows rapidly (Meyer 1954).

Himalayan white pine has been planted in all continents outside its range. In the United States, individual specimens are found in all northeastern states, but they are most common in the area near Philadelphia, Pennsylvania (Wright 1958). In comparison to P. strobus, the growth of Himalayan white pine is faster at Rochester, N.Y., but slower at the Pack Forest in New York.

A report by Lemmien and Wright (1963) indicates that Himalayan white pine can grow as far north as southern Michigan. A few 32-year-old trees were 52 feet tall and had 12-inch diameters breast high. In comparison to P. strobus, they had smaller heights, but slightly larger diameters.

In Maryland, the author has registered small plantings of P. griffithii at about 20 locations. The three largest Himalayan white pines in the U.S.A. grow near Catonsville, Maryland. At age 38 they were about 75 feet tall, had straight stems, and were healthy in all respects. Natural reproduction at this location indicate that Himalayan white pine is able to reproduce when planted outside its natural range.

Wright and Gabriel (1959) reported that some trees were injured and killed in Philadelphia during a severe winter of 1933-34; at the George Landis Arboretum in New York, it is classified as "not successful" because young seedlings are killed by early frost (personal communication with Fred Lape, Director); while in Highland Park of

Rochester, New York, two specimens of Himalayan white pine grew very well. Himalayan pine suffers from attacks by the white pine weevil (Pissodes strobi Peck) in southern Connecticut (Bailey 1955), and Genys (1977) reported from Maryland that P. griffithii can be attacked by Nantucket pine tip moth (Rhyacionia frustrana Comstock). One of the best traits of this species is its high degree of resistance to white pine blister rust (Cronartium ribicola Fischer) (Heimburger 1956, Wright and Gabriel 1959).

In the present study, Himalayan white pine was represented by 11 seed sources from native stands and 10 from trees planted in the United States and Italy. The major objectives were:

(1) To determine the nature and extend of genetic variation in Himalayan white pine, and

(2) to determine the potential for selection of frost-hardy Himalayan white pine for planting in northern regions of the eastern United States.

MATERIALS AND METHODS OF STUDY

Twenty-one seedlots for this experiment were acquired in 1961 and 1962 from 11 native stands in Pakistan, India and Bhutan, and 10 trees planted in the United States and Italy. A random sample of 50 filled seeds from each lot was used for determination of 1000-seed weights (data code No. 6 in table 1).

Two experiments were conducted at the State Forest Tree Nursery in Harmans, Maryland (latitude 39°07'N, longitude 76°45'W). This site is in plant hardiness zone 7 with average annual minimum temperatures ranging from 0° to 10°F, a frost-free period lasting from 180 to 210 days, and the first fall frost occurring between October 30th and November 10th.

The first experiment in the nursery was started on November 19, 1962. Twenty seed lots were used to establish a 4-times replicated, randomized block experiment using 2-foot rows as plots. Seedlings were spaced 2 inches apart within rows and 6 inches between rows. Bulk plots of seedlings were also grown for field plantings. The replicated plots were used for studies of coded characteristics as follows:

(8) Needle length

(9) The day of the year when 50 percent of 1-year-old seedlings set terminal buds,

(11) The percent of 1-year-old trees exhibiting secondary growth (resuming growth after terminal buds were once formed),

(12) The 2-year height based on the plot averages of the four largest seedlings representing four quarters of row-plots, and,

(13) The 2-year diameter above groundline on the three largest sample trees, dug from each plot before planting.

A second similar experiment in the nursery was established in spring 1967, using seed-lots of 15 sources. This time, two characteristics were studied:

(10) The percent of 2-year-old trees with terminal buds on October 24, 1968, and,

(14) The 2-year heights.

During the springs 1965, 1966 and 1967, the trees seeded in November 1962 were planted in Maryland (on 3 sites), Michigan (on 3 sites), Illinois (on 2 sites), Maine (1), New York (1), and North Carolina (1). Site descriptions, the age of stock and experimental designs of these plantations are given in the accompanying list.

Survival and heights were studied at various ages. Data in this report were based on 10- and 12-year-old trees. Heights are included for the two plantations in Maryland which had sufficient numbers of trees. White pine weevil (Pissodes strobi) was studied in the New York and North Carolina plantations.

Analysis of variance was based on the randomized block design. The least significant differences (L.S.D.) were determined by the Duncan's multiple range test. Character correlation and the correlations between measured characteristics and geographic data were also determined; only significant correlations will be discussed in the text.

RESULTS

Characteristics of Seedlings in the Nursery

Seed weights

Weights of 1000 filled seeds of P. griffithii from 9 stands and 10 individual tree collections varied from 36 grams (collected from a planted tree in Catonsville, Md.) to 77 grams (collected in Oxford, Md.). There was less variation among the seed lots from the native stands (table 1). For the stand collections, the correlation between the seed weights and eastern longitude was $r = .-73^{*}$ (d.f. = 6). Seeds from West Pakistan tended to be heavier than those from India and Bhutan.

List of research sites, experimental design, dates of planting, and age of stock

Maryland, Carroll Co., Piedmont Plateau, near Liberty Lake; 39°23'n; 76°54'W; elev. 490 ft; property of City of Baltimore; a former farm field; fertile, good drainage.	March, 1965; 2-0 stock; 4-tree square plots; spacing 7 ft x 7 ft; 21 populations; replicated in 4 blocks; measured at age 10.
Maryland, Anne Arundel Co., coastal plain, near Annapolis; 39°02'N; 76°24'W; elev. 15 ft; property of State of Md.; poor drainage; fertile soil.	April, 1965; 2-0 stock; 4-tree square plots; spacing 7 ft x 7 ft; 21 populations, replicated in 4 blocks; measured at age 10.
Maryland, Garrett Co., mid-Appalachian Mtns., Pleasant Valley Research Center; 39°3'N, 79°14'W; elev. 2600 ft; rocky and shallow soil; growing season about 130 days, cold winters.	April, 1965; 2-0 stock, spaced at 7 ft x 7 ft; 4-tree plots; 21 populations; replicated in 4 blocks. Last measurements made in Fall 1974.
Michigan, Kalamazoo Co., Kellogg Farm, near Hickory Corners; elev. 700 ft, slopes 3 to 30%; brush killed by 2,4,5-T; soil sandy loam to loamy sand.	April 9, 1966; 2-1 stock; spaced 8 ft x 6 ft; 4-tree row plot, 11 strains, replicated in 4 blocks. Last measurements made on July 17, 1974.
Michigan, Cass Co., Fred Russ Forest; elev. 700 ft; previous corn field, moved, and 3-foot strips sprayed with aminotriazole.	March 3, 1966; 2-1 stock; 4-tree plots; 10 strains replicated in 4 blocks. Last measurements on July 17, 1974.
Michigan, Ingham Co., Tree Research Center; elev. 700 ft; moist site, moderately well drained silt soil.	April 9, 1966; 2-1 stock; spaced 8 ft x 6 ft; 4-tree row plots; 12 populations; 2 blocks. Last measurements: early summer 1974.
Maine, Penobscot Co., near Olamon; state-owned land; 45°8'N, 68°35'W; elev. 200 ft; moderately well drained, 2 ft deep, sandy loam soil.	May 1967; 2-2 stock; originally 16 populations; 8 blocks; only 4 blocks were complete.

List of research sites, experimental design, dates of planting, and age of stock. cont.

New York, Warren Co., Heiberg Memorial Forest, north of Warrensburg.	April 1965; 2-0 stock; 15 populations; 4-tree square plots in 4 blocks.
Illinois, Henderson Co., Oquawka; level, loamy sand soil, dry in summer.	April 16, 1965; 2-0 stock; 6 populations in 4 blocks, represented by 4-tree plots; trees spaced at 7 ft x 7 ft. in 1974 only 3 blocks available for analysis.
Illinois, Edwards Co., West Salem; level, silty loam site. Simazine was applied after planting.	April 16, 1965; 2-0 stock; 6 populations in 5 blocks represented by 4-tree plots; trees spaced at 7 ft x 7 ft.
North Carolina, Madison Co., near Mars Hill, north of Asheville, southern slopes of south Appalachian Mtns.; shallow, rocky soil.	April, 1965; 2-0 stock; 15 populations, replicated in 4 blocks; 4-tree square plots; spacing 6 ft x 8 ft; some trees were currently injured by fire when measured in Spring 1975.

Leaf lengths

Leaf lengths in 6 of the provenance collections and 6 families ranged from 8.9 cm (No. 589 from a tree planted in Oxford, Md.) to 12.3 cm (No. 282 from Pakistan). Five out of six populations from the native range had longer needles than those from the planted trees.

Setting of terminal buds

Setting of terminal buds in the fall was studied twice in the nursery. Twelve populations (6 representing provenances and 6 single tree families) studied in fall 1964 set their terminal buds during the period from October 23 to November 7. Populations with the latest budset originated from planted trees (Nos. 589, 588, and 596). Populations from native stands showed only a 5-day variation in bud set date. Date of bud set was negatively correlated with the leaf length ($r = -.72^{**}$ d.f. = 10) suggesting that populations with short leaves set their buds earlier.

On October 24, 1968, twelve populations (10 representing provenances and 2 single tree families) of 2-year-old trees had different numbers of trees with terminal buds, ranging from only 2 percent (No. 721 from Bhutan) to 32 percent (No. 762 from Rochester, N. Y.). These two populations, showing extreme values in bud set, also showed extreme values in their survival rates in cold regions. All trees of population No. 721 set buds late and all died; trees from Rochester set their buds early and showed the best survival. The percent of trees with buds was negatively correlated with eastern longitude ($r = -.85^{**}$ d.f. = 7).

Secondary growth

In the six provenances and 6 families studied, the tendency for secondary growth (starting growth after primary terminal bud was formed) varied significantly (table 1). Some populations had as few as 23 percent of trees with secondary growth (No. 282 from Pakistan), while others had 74 percent (No. 592 from Annapolis, Md.). This characteristic showed no significant correlation to geographic data, and, contrary to expectation, it was not related to the survival rates in the northern regions.

Two-year height growth

In 1964 the 2-year-old Himalayan white pines (9 provenances, 6 families) averaged 12.2 cm in height. Variation among populations ranged from 84 percent (Nos. 588 and 596 from planted trees in Maryland) to 119 percent (No. 589 from trees planted in Maryland) of the mean for all populations. In 1968 (10 provenances, 2 families), 2-year heights averaged 8.5 cm, and differences among populations

Table 1.--Origin and growth data of different populations of Himalayan white pine (*Pinus griffithii*) studied in Maryland (at the State Forest Tree Nursery) in 1963-64, and 1967-68 (2-year heights)

Code: (2)	(3)	(4)	(6)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
Seed : Seed origin-country			1000 :		Bud set		Trees :	Two-year data		
lot :Longi-	Lati-	Alti- :	seed :	Needle :	Day of	10/24 :	w/2nd :	1964		1968
no. :tude	tude	tude :	weight :	length :	year	trees :	growth :	Height	Diam.	Height
Mean height or diameter in cm:								12.2	.42	8.5
		Feet	Grams	Cm		Percent	Percent	---- Percent of mean----		
Pakistan										
719 67°30'	29°00'	7500	65	--	--	26	--	107	--	107
756 72°22'	34°04'	7500	--	--	--	--	--	--	--	--
720 73°12'	33°30'	7000	51	--	--	13	--	92	--	100
103 73°15'	35°38'	6500	53	11.6	302	17	37	99	98	104
282 73°20'	34°10'	7900	56	12.3	304	9	23	111	95	102
280 73°20'	34°10'	7600	59	11.2	302	14	51	117	117	107
281 73°30'	34°	7500	66	11.6	303	12	54	110	110	119
India										
296 77°14'	32°15'	6200	51	10.5	304	10	35	102	100	95
771 78°	31°	7200	--	--	--	5	--	102	--	123
297 --	--	7000	46	11.7	307	17	65	111	107	110
Bhutan										
721 86°06'	27°42'	7500	46	--	--	2	--	--	--	94
Planted trees										
106 Italy			55	10.2	307	--	37	102	100	--
586 Catonsville,Md.(No.1)			62	--	--	--	--	--	--	--
587 Catonsville,Md.(No.2)			63	10.8	305	--	36	88	--	--
588 Catonsville,Md.(No.3)			36	10.8	308	--	39	84	86	--
589 Oxford,Md.(No.1)			77	8.9	311	--	45	119	102	--
592 Annapolis,Md.(No.2)			71	10.0	306	--	74	101	95	--
596 Solomons,Md.			58	9.6	308	6	38	84	88	80
598 Washington,D.C.(No.1)			74	--	--	--	--	--	--	--
641 Easton,Md.			72	--	--	--	--	--	--	--
762 Rochester, NY			49	--	--	32	--	--	--	83
The least significant difference at 0.05 level			--	2.7	ns	16	38	30	27	19

varied from 80 percent (No. 596 from Solomons, Md.) to 123 percent (No. 771 from India) of the mean. Two-year heights of the same populations, studied in 1964 and 1968, showed a significant positive correlation ($r = .63^{*}$ d.f. = 8).

In 1964 (6 provenances, 5 families), basal diameters averaged 0.42 cm and differences among populations ranged from 86 percent (No. 588 from Catonsville, Md.) to 117 percent (No. 280 from Pakistan). Diameters were significantly correlated with the 2-year heights measured in 1964 and 1968 ($r = .81^{**}$ d.f. = 9 and $.78^{*}$ d.f. = 5, respectively).

Characteristics after Planting

Data on survival in three plantations, heights in two plantations, and white pine weevil attack in two plantations are summarized in table 2.

Survival

Survival rates of Himalayan white pine varied in different climatic regions, and were related to the origin of seed source.

Survival in Maine, New York, Michigan, Illinois and Garrett County, Maryland, of Himalayan white pine was very poor at all sites located in plant-hardiness zones 3, 4 and 5, where the average annual minimum temperatures are -5°F or lower. In all this region, more than half of the populations had 0 percent survival, and none of the populations had more than 31 percent of trees living. Among the provenances showing best survival (13 percent) were Nos. 280, 282 and 719 from West Pakistan. Of the single tree families, Nos. 106, 586 and 762 were among the best. Trees from higher elevations showed somewhat better survival than those from lower elevations. In the Garrett County, Md. plantation, trees with early budset tended to have higher survival rates.

In Maryland plantations located in the Piedmont Plateau (Carroll County) and Atlantic Coastal Plain (Anne Arundel County) within plant hardiness zone 6, Himalayan white pine survived as well as eastern white pine. In Carroll County, survival rates among the 11 provenance collections and 10 families ranged from 0 percent (No. 721 from Bhutan) to 100 percent (No. 592 from Annapolis, Md.) and about two-thirds of the populations had survival rates exceeding 50 percent). Survival rates in this plantation were positively correlated with survival rates in the Anne Arundel County, Md. ($r = .72^{**}$ d.f. = 19) plantation, and negatively correlated with longitude ($r = -.69^{*}$ d.f. = 9). In Anne Arundel County, survival rates among populations ranged from 13 percent (No. 721 from Bhutan) to 100 percent (No. 587 from Catonsville, Md.), and 19 of 21 populations had survival rates greater than 50 percent. In this plantation, survival rates were also negatively correlated with longitude of seed source ($r = -.85^{**}$ d.f. -9) (table 2).

Table 2.--Survival and growth rates of different populations of Himalayan white pine, *Pinus griffithii*, in eastern United States (some data on attack by white pine weevil, *Pissodes strobi*, are also included)

Code	(2)	(3)	(4)	(20)	(21)	(22)	(23)	(24)	(26)	(27)
				Survival			Height		Weevil attack*	
Seed lot no.	Seed origin-country: Longitude	Latitude	Altitude	Carroll County Md.	Anne Arun. County Md.	North Carolina	Carroll County Md.	Anne Arun. County Md.	New York	North Carolina
Age of studied trees (yrs)				10	10	12	10	10	-	-
Mean height $\bar{x}$ (feet):				-	-	-	5.8	5.1	-	-
			Feet	Percent			Percent of mean		Number	
Pakistan										
719	67°30'	29°00'	7500	50	81	25	115	114	0/1	1/4
756	72°22'	34°04'	7500	94	88	--	89	103	--	--
720	73°12'	33°12'	7000	44	81	--	--	77	--	--
103	73°15'	35°38'	6500	63	63	19	89	91	--	3/3
282	73°20'	34°10'	7900	38	67	6	--	112	0/2	0/1
280	73°20'	34°10'	7600	75	75	31	124	97	0/2	2/5
281	73°30'	34°	7500	63	88	31	104	105	0/1	3/3
India										
296	77°14'	32°15'	6200	44	44	6	--	--	--	1/1
771	78°	31°	7200	56	75	6	123	138	--	1/1
297	--	--	7000	44	56	19	82	105	0/1	3/3
Bhutan										
721	89°06'	27°42'	7500	0	13	0	--	--	--	--
Planted trees										
106	Italy			56	67	25	91	125	1/3	3/4
586	Catonsville, Md. (No. 1)			67	94	--	70	97	1/3	--
587	Catonsville, Md. (No. 2)			88	100	13	107	108	--	1/3
588	Catonsville, Md. (No. 3)			67	75	13	--	84	--	0/2
589	Oxford, Md. (No. 1)			50	81	25	--	77	--	2/4
592	Annapolis, Md. (No. 2)			100	75	31	84	76	--	2/5
596	Solomons, Md.			63	88	0	--	94	0/1	--
598	Washington, D.C. (No. 1)			56	81	--	130	119	--	--
641	Easton, Md.			44	75	--	130	138	--	--
762	Rochester, N.Y.			75	88	--	91	68	0/3	--
The least significant difference at 0.05 level				68	56	ns	70	61	--	--

* trees attacked by *Pissodes strobi* (left): trees studied (right).

The North Carolina plantation is located in the transition region of plant hardiness zones 6 and 7. On this site, Himalayan white pine (9 provenances, 6 families) showed a better survival than in the northern regions, but worse than in Maryland's hardiness zone 6. Survival rates ranged from 0 percent (Nos. 721 from Bhutan, and 596 from Solomons, Md.) to 31 percent (Nos. 280 and 281 from Pakistan and No. 592 from Annapolis, Md.). Survival in North Carolina was correlated with survival in Carroll County, Md. ($r = 0.52^*$ d.f. = 13) and was correlated with diameters of 2-year-old trees ($r = .64^*$ d.f. = 9).

Height growth in plantations

Heights are only reported for the two plantations in Maryland (Carroll and Anne Arundel Counties) with good survival.

In Carroll County, heights of 10-year-old trees averaged 5.8 feet. Heights of different populations and families ranged from 70 percent to 130 percent of the plantation mean. The slowest growing family was from planted tree No. 1 in Catonsville, Maryland (No. 586). The fastest growing families originated from planted trees in Washington, D.C. (No. 598) and Easton, Md. (No. 641), followed by populations from Pakistan (No. 280) and India (No. 771). Heights in this plantation were significantly correlated with diameters of 2-year-old trees ($r = .82^*$ d.f. = 4) and with heights of 10-year-old trees in Anne Arundel County, Md. ($r = .60^*$ d.f. = 12).

In Anne Arundel County, 10-year-old trees had an average height of 5.1 feet. Heights of different populations and families ranged from 68 percent to 138 percent of the plantation mean. Slowest growing were the families from Rochester, N.Y. (No. 762), Annapolis, Md. (No. 592), Oxford, Md. (No. 589), and the population from Pakistan (No.720). The fastest growing were again the family from Easton, Md. (No. 641), and the populations from India (No. 771), and Italy (No. 106). Heights in this plantation were significantly correlated with latitude ($r = .75^*$ d.f. = 6).

In summary, the best families were from planted trees No. 641 in Easton, Maryland, and No. 598 in Washington, D.C., and from native stands No. 771 in Pradish, India, and No. 719 in Shogran, West Pakistan. With the exception of the populations No. 280 from West Pakistan and family No. 106 from Italy, rankings were rather similar at the two sites.

Reliability of early growth data

If trees were selected for rapid growth on the basis of 2-year heights in 1964, populations No. 589, 280, 282 and 297 would be included. However, none of these populations were among the four best at age 10. In fact, 2-year heights of the first experiment

(1963-1964) showed no relationship to 10-year heights in the Maryland plantations (r = .20 and .14). Much more reliable for prediction of 10-year heights were the 2-year diameters (r = .82* d.f. = 4 and r = .26 d.f. = 8) and 2-year heights of seedlings studied in 1967-1968 (r - .57 d.f. = 5, and r = .45 d.f. = 8).

Attack by white pine weevil

White pine weevil (Pissodes strobi) was studied in New York and North Carolina (table 2). In New York, out of 17 surviving trees, two (12 percent) were attacked by white pine weevil. In North Carolina, weevil attack was more extensive, and out of 39 trees, 22 (56 percent) were injured. In this plantation, populations from low elevations (like No. 103) included more trees attacked by white pine weevil than those from higher elevations (like No. 282), and the correlation of the numbers of attacked trees with the elevation of the seed-source was r = -.84** d.f. = 6

Cone production

In North Carolina, at age 12, about 10 percent of Himalayan white pine had cones. The most pronounced cone producer was population No. 280 from Pakistan. Out of 4 trees studied, 3 had cones. In general, at this early age, P. griffithii had fewer cones than P. strobus (20 percent of trees with cones).

Comparison of Himalayan White Pine with Eastern White Pine

In comparison to P. strobus, Himalayan white pine had heavier seeds, and when growing on the same sites longer leaves, smaller 2-year heights, larger 2-year diameters, more trees with secondary growth, and set terminal buds later in the fall. When planted in the field and studied for 8 or 10 years, Himalayan white pine had significantly greater mortality than P. strobus in Maine, New York, Michigan, Illinois, Garrett County, Md., and North Carolina. It survived nearly as well as P. strobus in Carroll County, Md., and showed a similar survival rate in Anne Arundel County. In Maryland's Piedmont Plateau and Coastal Plain, Himalayan white pine had smaller 10-year heights than eastern white pine. In North Carolina, both species had similar percentages of trees attacked by Pissodes strobi, Himalayan white pine had fewer trees with cones. While the characteristics listed above were distinctly different, in other measurements one species were within the range of the other species. Such overlapping ranges at variation were in numbers of cotyledons, time of bud-set, and growth rates of different populations.

DISCUSSION

Results on seed weight, date of bud-set, percent of trees with secondary growth, and growth rate of 2-year-old trees are mainly of basic interest, while the survival rates of different populations planted in the northern regions of eastern United States are of more practical importance. Also important are the data on variation in growth rates of 10-year-old trees.

Studies on survival rates in various hardiness zones lead to identification of several general trends. In the northern plantations, except in Illinois, populations Nos. 280, 281 and 719 from Pakistan were most promising. In all plantations, population No. 721 from Bhutan failed completely, and population No. 762 from a planted tree in Rochester, N.Y. showed the best or the second best survival. Correlation analysis suggests that survival rates were negatively correlated with the eastern longitude and the date of bud-set, and positively correlated with the elevation of seed source. Apparently, best survival rates can be expected from the seed sources from western longitudes, higher altitudes, and populations setting terminal buds early in the fall. Also, good survival can be expected from some progenies originating from planted trees in northern regions (like Rochester, New York).

Contrary to many instances in other pines, the growth rates of *P. griffithii* showed a positive relationship with northern latitude and elevation of the seed source. Also, heights showed positive relationships with needle lengths and diameters of 2-year-old seedlings, and a negative correlation with day of budset and the percent of trees with secondary growth.

If selection of seed sources for rapid growth are based on these current results, most likely trees planted in Easton, Maryland (No. 641) and Washington, D.C. (No. 598), would be recommended for use as seed sources. Among the populations from the native region, most promising were No. 771 from India for warmer regions and Nos. 719, 282 and 280 from Pakistan for colder regions.

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THE BIONOMICS OF THE COTTONWOOD LEAF BEETLE, *CHRYSOMELA SCRIPTA* FAB., ON TISSUE CULTURE HYBRID POPLARS[1]

T. R. Burkot and D. M. Benjamin[2]

ABSTRACT.--Tissue culture methods are applied to poplars of the *Aigeiros* group in attempts to overcome premature decline thought to be associated with viral infections. Hybrid selections from such cultures outplanted in 1975 at the F. G. Wilson Nursery in Boscobel, Wisconsin subsequently were severely infested by the Cottonwood Leaf Beetle, *Chrysomela scripta* Fab. Beetle populations built up rapidly in spring 1977 and severe defoliation occurred; an average of 45% of the terminal buds were destroyed and 73 percent of the tips of a preferred clone were fed upon. Younger foliage appeared preferred and certain clones sustained more feeding in field and laboratory studies. Plants receiving greater amounts of overhead irrigation were least defoliated. Rapid increases in the numbers of the insect in Wisconsin are reflected in the following facts. The Cottonwood Leaf Beetle has a rapid life cycle (19 days at 27°C). Consequently, the insect undergoes 4-5 generations per year in Wisconsin where it exhibits a high fecundity of approximately 510 eggs. Finally, because of the larval defensive secretion, salicylaldehyde, the insect has few natural enemies; mainly predatory pentatomids on larvae and adults, and *Shizonotus latus* Walker, a pupal parasite.

Black poplars of the *Aigeiros* group maintained by the University of Wisconsin were undergoing premature decline, thought to be associated with viral infections. In an attempt to culture poplars for high yields, heat treatment followed by tissue culture methods were applied to the apical meristems to eliminate viruses. Heat treatments allow the trees to outgrow viruses and shoot-tip culture capitalizes on the absence or

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lowered concentration of viral particles in the apical meristems (Hollings 1965). Such clonal propagation might allow genetically superior traits to be transferred by eliminating the chance of gene recombination occurring via a sexual cycle. However, a wide range of variation for height, number of branches, and leaf traits were discovered among subclones. The basis of this variation is as yet unknown, but the more obvious possible causes include incomplete elimination of viral infection, gene mutation, and chromosomal instability (Lester and Berbee 1977).

Hybrid selections of these subclones were outplanted in 1975 at the F. G. Wilson Nursery at Boscobel, Wisconsin, and subsequently were severely infested by the Cottonwood Leaf Beetle, Chrysomela scripta Fab. The defoliator, although it rarely reaches epidemic numbers in natural stands of Populus, Alnus, and Salix, has become an established annual problem in the monoculture cottonwood plantations of the South, as well as in the nurseries of Wisconsin.

In Mississippi, the insect increased rapidly in numbers because of its high natural fecundity of 823 eggs, a short life cycle, and as many as seven generations per year (Head and Neel 1973). In addition, the Cottonwood Leaf Beetle has few natural enemies because of its defensive secretation, salicylaldehyde, which is exuded by eversible glands on the thorax and abdomen (Wallace and Blum 1969).

We are studying the bionomics of the Cottonwood Leaf Beetle in Wisconsin to develop means of preventing the insects from reaching numbers that result in severe defoliation and destruction of apical tips. In addition, since the tissue cultured hybrid poplar subclones showed variability in vigor and other characteristics, we expect that there would be variability in resistance to insect attack. We will try to develop a method of screening clones for relative resistance.

METHODS

In May, 1977, three Latin squares were established at the F. G. Wilson Nursery at Boscobel, Wisconsin: each square consisted of 25 trees, five trees from each of four of tissue culture Aigeiros subclones and five trees from a tissue culture Lombardy clone. The three Latin squares made up one replicate. A second replicate of these three Latin squares was planted at the University Experimental Farms in Arlington, Wisconsin, in May, 1977. Twice weekly, a count was made of all eggs, larvae, pupae, and adults of the Cottonwood Leaf Beetle on each of the trees in the three Latin squares at the Arlington and Boscobel sites. In addition, a field plot of 1512 tissue culture poplars consisting of six subclones planted in 1975 at the F. G. Wilson Nursery at Boscobel, Wisconsin, served as a third site for biological observations of the Cottonwood Leaf Beetle. Eggs, larvae and pupae were also collected from this field plot and reared in the laboratory for

parasites. A record was made of predators seen on the trees. These data later will be incorporated into life tables.

At the end of each generation of the Cottonwood Leaf Beetle, a count was made of all the host apical tips destroyed in the plots to evaluate the economic damage and relative change in insect numbers.

Leaves on which eggs were laid were transferred to environmental chambers at different temperatures (10°C, 17°C, 21°C, 23°C, 27°C, 28°C) to determine the effects of temperature on developmental times and mortality; all rearing occurred at an 18-hour photoperiod.

Virgin males and females were paired and caged in 21.5 cm x 6.7 cm x 5.8 cm plastic boxes with foliage and placed in an environmental chamber at 27°C and 18 hrs of light. Daily counts were made of eggs laid to determine the average fecundity.

The number of larval instars was determined by measuring the head capsule widths of ten larvae daily throughout their development.

Adult preferential feeding tests were carried out by offering a caged male and female a choice of one leaf from each of two different subclones. Leaves of the approximate same size and relative position on their respective trees were used in each replicate. The turgor of the leaves was maintained by keeping the petioles of the leaves submerged in a vial of water plugged at the top with cotton. The tests were run for a week at 27°C, 18 hrs light. The area of the leaves was determined before and after feeding by using a plane polarimeter. The relative feeding preference by adult beetles on mature versus immature foliage from the same clone were likewise tested.

RESULTS

In Wisconsin, the Cottonwood Leaf Beetle has 4-5 generations per year. In the laboratory the females exhibit an average fecundity of 510 ± 153 eggs. The insect has three larvel instars and a short life cycle of 19 days at 27°C; optimal development occurred at 23°C.

Field studies showed both the larvae and adults to be defoliators and to prefer to feed on the more tender young leaves near the apical tip. The first and second instars feed by etching the lower surface of the leaves while the third instars and adults may skeletonize the leaves and leave only the main ribs. In addition, the third instars and adults feed on the apical tips and destroy them. Laboratory studies revealed that of the clones tested for adult preferential feeding, only Aigeiros clone 13 showed a significant difference (at α=5% for 2-t-test) from the others; it was preferred by at least four times that of the standard. Immature foliage was likewise preferred by a

ratio of 33:1 over mature foliage. In the field plot of 1512 trees, an average of 45 percent of the apical tips were destroyed and 73 percent of Aigeiros clone 13 tips were fed upon.

Natural control agents were unable to maintain the population of the Cottonwood Leaf Beetle on the Boscobel plot in the second generation at a low economic level: Shizonotus latus Walker parasitizes 3.2 percent of the pupae while Coleomagilla maculata DeGreer consumes 12.4 percent of the eggs. The larval predators such as the lacewings, syrphids, and the pentatomids, Podisus maculiventris Say and Perillus bioculatus Fab. were unable to maintain the population of the Cottonwood Leaf Beetle at an acceptable sub-economic damage level. The amount of feeding damage sustained in the Boscobel nursery was inversely related to the amount of water received via an overhead sprinkler system.

On the Arlington replicates; the Cottonwood Leaf Beetle population was too low to evaluate meaningfully except for making biological observations.

DISCUSSION

Several facets of the Cottonwood Leaf Beetle's biology enhance its prospects for being a continuing problem in Populus nurseries in the Lake States. First, is its high fecundity of 510 eggs. Secondly, is its short life cycle which requires 19 days from adult at 27°C. Thirdly, the insect is multivoltine, having 4-5 generations per year in Wisconsin.

A larval defensive secretion repels insect predators and thus appears to restrict the kinds of the biotic control agents that can successfully attack the insect to pentatomids, syrphids and lacewings. Neither the egg predator, Coleomagilla maculata, nor the pupal parasite, Shizonotus latus, can reduce the Cottonwood Leaf Beetle to an economically acceptable level. This enables the beetle to increase rapidly in population. Abiotic factors, notably temperature, seem to mainly control the rapidity of increase. Watering from overhead sprinkler systems also limits the destructiveness of the beetle by knocking the adults off the trees. The more water falling on a plant, the less likely it is that an adult will feed there and oviposit. However, even temperature and overhead watering are unable to prevent the insect from reaching such numbers that an average of 45 percent of the apical tips are destroyed and 73 percent of the tips of the preferred clone are fed upon as at Boscobel.

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HERITABILITY CONSTRUCTION FOR PROVENANCE AND FAMILY SELECTION

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ABSTRACT.--Concepts and procedures for heritability estimations through the variance components and the unified F-statistics approach are described. The variance components approach is illustrated by five possible family selection schemes within a diallel mating test, while the unified F-statistics approach is demonstrated by a geographic variation study. In a balance design, the use of the heritability formula $h^2 = 1-(1/F)$ is recommended. The F value is the same computed F value used in the analysis of variance for testing quality of genetic units.

Heritability is defined as "degree to which a character is influenced by heredity as compared to environment" (Snyder 1972). Although the concept holds in a general sense, heritability estimates vary with the way they are calculated. Heritability can be calculated in four ways: (1) parent-offspring correlation estimates degree of resemblance between parents and offspring; (2) regression estimates the selection differential in offspring as dependent on the selection differential in the parent; (3) intraclass correlation estimates the degree of resemblance among individuals within a genetic classification, and (4) genetic gain divided by selection differential estimates the efficacy of selection. These four methods are interrelated and may be used interchangably, but in certain cases adjustments should be made (Franklin 1974, Squillace 1974).

Heritability and gain estimates apply only to the experiments from which they were obtained. Furthermore, the selection differentials and heritabilities must apply to the same things, which may be individual trees, or family means (Wright 1976). Although single

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tree heritability and family heritability are formulated in most textbooks (Lerner 1958, Falconer 1960, and Becker 1964), heritability for other genetic units such as stand, ecotype, family within stand, full-sib family within female parent, or family and site combination, are seldom available.

In this paper we show how to break down phenotypic variation and construct heritabilities according to the experimental design, mating system, and selection scheme that are being used. The purpose is to help persons to make heritability estimates clearer and more consistent. In addition, we propose a unified formula for heritability, $h^2 = 1-(1/F)$, where F is the computed value for testing the null hypothesis that all genetic units are equal. This heritability is useful for predicting genetic gain of the genetic units being tested and selected.

Variance Component Approach

The basic concept of heritability is the genetic variance among units divided by the phenotypic variance among units. Therefore, the procedure for constructing heritability from analysis of variance can be described as follows:

1. Compute phenotypic variance among units
2. Identify variance components
3. Assemble genetic variance
4. Divide genetic variance by phenotypic variance

h^2 in the Clonal Test

In the simplest case, a clonal test, each ramet is assumed to have received total and identical genetic information from the ortet. The phenotypic expression of a seedling is affected by the genetic effect of the ortet and the environmental effect, with the following model:

$$Y_{ij} = \mu + G_i + E_{ij}$$

where Y_{ij} = phenotypic expression of the jth ramet from the ith ortet

μ = population mean in the test

G_i = genetic effect due to the ith ortet (i = 1 to n)

E_{ij} = random independent and normally distributed environmental effect (i = 1 to n, and j = 1 to r)

The ANOVA table is shown in table 1, where Ve and Vg are variance components for environment and genetics, respectively.

Table 1.--Analysis-of-variance table for a completely randomized clonal test

Source	df	MS	EMS
Clone	n-1	$\frac{r}{n-1}\sum_{i=1}^{n}(Y_{i.}-Y_{..})^2$	Ve + rVg
Within	n(r-1)	$\frac{1}{n(r-1)}\sum_{i+1}^{n}\sum_{j=1}^{r}(Y_{ij}-Y_{i.})^2$	Ve
Total indiv.	nr-1	$\frac{1}{nr-1}\sum_{i=1}^{n}\sum_{j=1}^{r}(Y_{ij}-Y_{..})^2$	Ve + $\frac{(n-1)r}{nr-1}$ Vg

To construct heritability for clonal selection, we proceed as follows:

1. Compute phenotypic variance among clones and record as the value for Clone Mean Square.

2. Identify variance components: obtain Ve + Vg from Expected Mean Square (EMS).

3. Assemble genetic variance; solve two simultaneous equations for Ve and Vg and compute the value for rVg.

4. Divide the value for rVg by the Clone Mean Square.

Clonal heritability obtained is the form of $rVg/(Ve_2 + rVg)$, which is equivalent to the commonly accepted formula: $h^2 = Vg/(V\hat{g} + Ve/r)$ (Burton and Devane 1953).

h^2 in a Diallel Mating Test

Following the same procedure, we can construct heritability formulas suitable for various selection schemes after a diallel mating test.

Under the diallel mating scheme, every male mates with every female parent. For monoecious species, diallel mating includes a cross and reciprocal cross with every other tree. The statistical model is a two-way analysis of variance:

$$Y_{ijk} = \mu + M_i + F_j + MF_{ij} + W_{ijk}$$

where Y_{ijk} = the phenotypic value of seedling K produced from the ith male parent and the jth female parent (k = 1 to K).

μ = population mean

M_i = genetic effect from the ith male parent (i = 1 to I)

F_j = genetic effect from the jth female parent (j = 1 to J)

MF_{ij} = interaction between the ith male and jth female parents

W_{ijk} = experimental error (k = 1 to K)

In the conventional diallel analysis, where components are estimated from the ANOVA, heritabilities are estimated for male parents, female parents, and interaction (full-sibs). However, there are other selection schemes possible and each has its own heritability. Computation is possible by considering the sum of squares for the components in the diallel mating scheme.

The partitioning of variance can be facilitated by working with the individual expectation of sum of squares as listed in table 2.

Table 2.--Expectations of sum of square in a completely randomized diallel mating test

Line no.	Item	Interpretation	Coefficient for μ	σ^2_m	σ^2_f	σ^2_{mf}	σ^2_w
1	$\Sigma\Sigma\Sigma X_{ijk}^2$	Individual	IJK	IJK	IJK	IJK	IJK
2	$\Sigma\Sigma X_{ij.}^2/K$	Full-sib	IJK	IJK	IJK	IJK	IJ
3	$\Sigma X_{i..}^2/JK$	Half-sib in male	IJK	IJK	I K	I K	I
4	$\Sigma X_{.j.}^2/IK$	Half-sib in female	IJK	JK	IJK	JK	J
5	$X^2_{...}/IJK$	Correction term	IJK	JK	I K	K	I

Once we obtain the expected variance components for the sum of squares, we can proceed to the intermediate steps in formulating heritability:

A. The sum of squares for a "m" genetic unit within a "n" unit, SSQ m in n, is $SSQ_m - SSQ_n$. For example:

SSQ individual in plantation = SSQ individual - SSQ correction term

SSQ individuals within full-sib = SSQ individual - SSQ full-sib

SSQ full-sib within male parent = SSQ full-sib - SSQ half-sib in male

B. Heritability for differences among "m" units with "n" units is:

$$\frac{\text{Genetic Variance Component in SSQ m in n}}{\text{SSQ m in n}}$$

If we divide both the numerator and the denominator of the above formula by the degrees of freedom associated with the "m in n" unit, the numerator becomes genetic variance (Vg) and the denominator becomes phenotypic variance (Vp), and the result is the commonly accepted formula, $h^2 = Vg/Vp$. It is easier to work with SSQ than MSQ and ignore the degrees of freedom.

If we want to construct heritability for differences among full-sib families within male parent, we first determine the variance components within the SSQ full-sib in male. From lines 2 and 3 of table 2 we have:

$$\text{SSQ full-sib in male} = \text{SSQ full-sib} - \text{SSQ male}$$
$$= IK(J-1)\sigma_f^2 + IK(J-1)\sigma_{mf}^2 + I(J-1)\sigma_w^2$$

If we consider the first two terms as genetic contribution, the numerator in the heritability formula is:

$$IK(J-1)\sigma_f^2 + IK(J-1)\sigma_{mg}^2$$

and the denominator is simply SSQ full-sib in male, or all of its associate variance components.

If we further consider the genetic parameters in terms of additive genetic variance (Va) and dominance variance (Vd), we have $\sigma_f^2 = 1/4$ (Va), $\sigma_{mxf}^2 = 1/4$ (Vd) and $\sigma_w^2 = 2/4$ (Va) + 3/4 (Vd) + σ_e^2, and the numerator becomes:

$$IK(J-1)1/4(Va) + IK(J-1)1/4(Vd) + I(J-1)\{2/4(Va) + 3/4(Vd)\}$$

and the denominator becomes:

$$IK(J-1)1/4(Va) + IK(J-1)1/4(Vd) + I(J-1)\{2/4(Va) + 3/4(Vd) + Ve\}.$$

While the second consideration is more complete and academic, the first consideration is practical and useful in gain prediction. Heritabilities for family selection are therefore constructed on the basis of the first consideration (table 3).

Table 3.--Heritability computation for family selections

h^2 for differences among "m" (m)	h^2 for differences within "n" (n)	Denominator obtained from lines	Variance Components in the Denominator: Genetic (also used in the numerator) Coefficient for σ^2_m	Genetic Coefficient for σ^2_f	Genetic Coefficient for σ^2_{mf}	Error Coefficient for σ^2_w
Full-sib						
	Plantation	2-5	JK(I-1)	IK(J-1)	K(IJ-1)	IJ-1
	Male	2-3	--	IK(J-1)	IK(J-1)	I(J-1)
	Female	2-4	JK(I-1)	--	JK(I-1)	J(I-1)
Half-sib						
Male	Plantation	3-5	JK(I-1)	--	K(I-1)	(I-1)
Female						
	Plantation	4-5	--	IK(J-1)	K(J-1)	(J-1)

In short, the generalized procedure for constructing heritability from analysis of variance is modified as follows:

1. Compute sum of square among genetic units as the denominator.
2. Identify variance components which will have contributed to the genetic gain.
3. Assemble these contributing variance components with their coefficients as the numerator.

The $h^2 = 1-(1/F)$ Approach

The basic concept of this unified formula is that all sources of variance within a genetic classification are considered as error as far as predicting gain from selection among such classification is concerned. For example, in a half-sib progeny test, 3/4 of the additive genetic variance and all of the dominance genetic variance present within family are useless for mother tree selection and are consequently included in the error term.

The F value for testing equality of means is a variance ratio: among-group variance divided by within-group variance. Therefore, the formula $h^2 = 1/(1/F)$ is:

$$h^2 = 1-1/(\text{MS group}/\text{MS error})$$

$$= \frac{\text{MS group - MS error}}{\text{MS group}}$$

$$= \frac{\text{Phenotypic Variance - Error Variance}}{\text{Phenotypic Variance Among Groups}}$$

$$= \frac{\text{Genetic Variance Among Groups}}{\text{Phenotypic Variance Among Groups}}$$

The steps in constructing this heritability are simply:

1. Calculate the F value suitable for testing the quality among groups.

2. Insert the computed F value and solve for $h^2 = 1-(1/F)$.

Most geographic variation studies fit the nested model and are useful here as an example. Stands (S_{ij}) are nested in different regions (R_i). Within-stand variation is considered to be environmental random error (E_{ijk}). We can express the model as:

$$Y_{ijk} = \mu + R_i + S_{ij} + E_{ijk}$$

The analysis of variance table is as follows:

Table 4.--Analysis of variance table for a nested design

Source	: df	: MS	: F	: EMS
Region	r-1	MS_r	MS_r/MS_s	$\sigma_e^2 + n\sigma_s^2 + sn\sigma_r^2$
Stand/Region	s-r	MS_s	MS_s/MS_e	$\sigma_e^2 + n\sigma_s^2$
Error	rsn-s	MS_e		σ_e^2
Total	rsn-1			

To compute heritability for regional difference, the F value to be used in the formula is obtained from MS_r/MS_s. By the same token, to compute heritability for stand difference within region, the F value is MS_s/MS_e.

Fixed and Random Models and Corresponding Heritability

Analysis of variance models may be classified as fixed, random, or mixed effect models. An effect is considered to be fixed if the researcher is interested only in the various levels of treatment used in his experiment. On the contrary, an effect is considered to be random if the researcher is interested in the population from which the treatments were drawn. The mixed effect model includes some treatments in fixed effects and some in random effects. An example using a multiplantation multiyear half-sib progeny test under various assumptions will help to interpret the heritability formula (table 5). For fixed effects the variance component represents variance among treatments in the experiment. For

random effects the various components represent variance of the population from which the sample of treatments were drawn.

I. Fixed Effect: Mother Tree. Random Effect: Site and Year.

In this case we would be interested in selecting mother trees that would perform well on an average site through the years. The F test for mother tree is M/(MS + MY - MSY) (Kempthorne 1967). Substituting the expected mean square into the heritability formula, we have:

$$h^2 = 1-1/F$$

$$= (M - MS - MY + MSY)/M$$

$$= \frac{Vm}{\frac{Ve}{nsy} + \frac{Vsym}{sy} + \frac{Vym}{y} + \frac{Vsm}{s} + Vm}$$

The interpretation of this formula is that all effects other than the mother tree are useless errors in selection.

Table 5.--Multiplantation and multiyear model for heritability computation (only the relevant EMS are listed)

Source	: df	: MSQ	: EMS
Site	s-1	S	
Year	y-1	Y	
SxY	(s-1)(y-1)	SxY	
Mother tree	(m-1)	M	Ve+nVsym+nsVym+nyVsm+nsyVm
MxS	(m-1)(s-1)	MS	Ve+nVsym +nyvSm
MxY	(m-1)(y-1)	MY	Ve+nVsym+nsVym
MxSxY	(m-1)(s-1)(y-1)	MSY	Ve+nVsym
Replication	sy(n-1)	R	
Error	sy(n-1)(m-1)	E	Ve

II. Fixed Effect: Mother Tree and Site. Random Effect: Year

In this case we would be interested in selecting mother trees that would perform well on a specific site. The F test is M/MY. So we have:

$$h^2 = 1 - 1/F$$

$$= (M-MY)/M$$

$$= \frac{\frac{Vsm}{s} + Vm}{\frac{Ve}{nsy} + \frac{Vsym}{sy} + \frac{Vym}{y} + \frac{Vsm}{s} + Vm}$$

Under this model both mother tree and mother tree x site interaction are genetic and useful, while error, mother tree x year x site and mother tree x year, are considered as non-genetic and useless.

III. Fixed Effect: Mother Tree, Site and Year

If the year effect is found to be related to some controllable factors (for example, amount of rainfall and pattern of rainfall can be simulated by irrigation), then we might be interested in selecting mother trees under a specific site and repeatable conditions. The F test in this model is M/E. So we have:

$$h^2 = 1 - 1/F$$

$$= (M - E)/M$$

$$= \frac{\frac{Vsym}{sy} + \frac{Vym}{y} + \frac{Vsm}{s} + Vm}{\frac{Ve}{nsy} + \frac{Vsym}{sy} + \frac{Vym}{y} + \frac{Vsm}{s}}$$

The interpretation would be that of all the mother tree effect and its interaction with site and year are genetic and useful for selection.

Discussion and Conclusions

For a given model, both approaches yield an identical estimate of heritability. When the experimental design is balanced, the $h^2 = 1-(1/F)$ approach is recommended. The procedure is simple and useful for predicting genetic gain for the units being tested. The F-value is readily available from the analysis-of-variance table used for testing the quality of genetic unit means. There is no need to compute variance components and their coefficients, no worry about negative components, and no doubt about what goes into the heritability formula. However, the model that is being used and its implications must be thoroughly defined, and the F-value should be significant at a certain level. For example, it would be in error in a one-parent progeny test to consider families as fixed effects and then apply heritability and genetic gain values to the parent population. The concept of model effects and the appropriate F-value are more common than the expected variance components in most introductory statistics books (Li 1964, Ostle 1964, Bliss, 1967, and Daniel

1974). It appears to be easier for tree improvement workers to find the appropriate F-ratio than to determine which genotypic x environmental interactions should be included in the error term of the heritability formula.

If the experimental design is not balanced, the task of formulating a valid F-value may be as difficult as solving variance components. In an unbalanced design, the F-ratio and consequently the heritabiility is only an approximate value. Most provenance tests can be handled as nested analysis of variance with unequal sample sizes. Sokal and Rohlf (1969) gave two good examples in computing variance components and the approximate test of significance based on a reconstituted mean square.

The $h^2 = 1-(1/F)$ concept regards any repeatable effect present among genetic units as inheritable. For example, when testing wind pollinated progenies, the mother-tree effect includes not only the additive genetic contribution of the mother tree, but also the contribution of the wind-borne pollen as well as the extra chromosomal inheritance. On the other hand, this formula disregards any genetic variance within units. The variance components approach, by defining genetic and non-genetic variances, can demonstrate explicitly the definition of heritability (that portion of phenotypic variance due to genetic effects). However, the $h^2 = 1-(1/F)$ approach is functional for predicting gain in family, stand, and ecotype units.

The $h^2 = 1-(1/F)$ approach is restricted to the units being tested and selected, whereas the variance components approach is more flexible in estimating other related heritabilities. For example, in a progeny test, heritability of individual differences, useful in predicting gain in mass selection, can be constructed only by the variance components method.

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ANALYSIS OF VARIANCE CALCULATIONS FOR IRREGULAR EXPERIMENTS[1]

Jonathan W. Wright[2]

ABSTRACT.--Irregular experiments may be more useful than much smaller regular experiments and can be analyzed statistically without undue expenditure of time. For a few missing plots, standard methods of calculating missing-plot values can be used. For more missing plots (up to 10 percent), seedlot means or randomly chosen plot means of the same seedlot can be substituted for missing plots, provided between-block differences are small. Whatever the number of missing plots (provided there is more than one plot per seedlot) or the size of the between-block differences, seedlot means and sums of squares can be estimated in terms of deviations from block means. The procedures for calculation of analysis of variance in terms of deviations are described. The procedures are also applicable to regular experiments.

Ideally, a forest genetic experiment should follow a regular design in which each seedlot is represented in an equal number of blocks in every plantation. However, perfect regularity is rarely possible. There are usually a few missing plots as the result of mortality. Also, many times planting stock is more limited for some seedlots than for others. When that happens, one can plan a regular experiment by reducing the numbers of families and replications, or one can go ahead and distribute each family to each replication as long as the planting stock lasts. If the first alternative had been followed, most NC-99[3] experiments would have been reduced in size by 90 percent. Most NC-99 members probably agree that the second alternative was more desirable even though it resulted in experiments with great irregularities.

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[3] NC-99 is an organization of University and federal forest tree geneticists. The group has conducted cooperative provenance and progeny tests in northcentral United States for almost 20 years.

This paper has two objectives. The first is to present relatively simple methods to analyze data from experiments with various degrees of irregularity. The second is to allay frequently encountered fears of irregular data when planning experiments that might lose much information if made perfectly regular.

In using these methods, one must remember that the resulting analyses are only as strong as the data base. One can devise calculation methods to allow for missing data, but the inferences can never be as strong as if the data were not missing.

STANDARD PROCEDURE FOR CALCULATING MISSING PLOT VALUES

With a randomized complete block experiment in which seedlot A is missing from block 1, the standard procedure for calculating a substitute value is as follows (from Sokol and Rohlf 1969):

(1) Calculate all seedlot sums, block sums, and the overall sum, omitting the missing plot in each case.

(2) Calculate a missing plot mean as follows:

$$\text{Missing plot mean} = \frac{Nb(\text{Sum seedlot A}) + Ns(\text{Sum block 1}) - \text{Overall sum}}{(Nb-1)(Ns-1)}$$

where Nb = number of blocks and Ns = number of seedlots.

(3) Using this substitute value, calculate the analysis of variance in the normal manner.

(4) Reduce the degrees of freedom for error by 1 for each missing plot.

If there is more than one missing plot, the first value must be calculated by using reasonable estimates for the other values, using the first value and reasonable estimates for all other values to calculate the second, etc. When there are many missing values, the preliminary estimates and calculated values may differ appreciably, in which case it may be necessary to redo some of the calculations.

This is the best way to calculate a missing-plot value, but is laborious if the number of missing plots is large.

USE OF SEEDLOT MEANS OR RANDOMLY CHOSEN PLOT MEANS

If the number of missing plots is small and there are minor differences among blocks, either of two simple procedures can be used. For each missing plot, one can substitute either (1) the mean for that seedlot, or (2) the mean of a randomly selected plot of the seedlot. In either case, reduce the degrees of freedom by 1 for each missing plot.

The first method changes seedlot means the least but the second is easiest to use with a computer.

The validity of these procedures can be checked by analyzing data from a completely regular experiment, eliminating some plots arbitrarily, substituting values for them, and re-calculating the analysis of variance. This has been done with several sets of data. Generally, there has been less than a 1 percent change in any mean square or F value as the result of substituting for up to 10 percent of the plot means.

Use of one of these simple substitution methods will normally cause less than a 2 percent change in the mean for an individual seedlot if only one or two plots per seedlot are missing and block means differ by less than 20 percent. Another calculation method may be preferable if there are more missing plots or large differences among blocks.

DISCARDING UNDER-REPRESENTED SEEDLOTS OR BLOCKS

If the missing plots are concentrated in a few seedlots or blocks, those seedlots or blocks can be eliminated to make the remaining data perfectly regular. This procedure is often satisfactory unless the incompletely represented seedlots are among the best.

ANALYSIS BASED UPON DEVIATIONS RATHER THAN TOTALS

Most students learn to do an analysis of variance by squaring the plot means, the seedlot sums, the block sums,etc. The analysis can be done, however, by working with the deviations of the plot means from the block means or seedlot means. By working with such deviations, one can avoid most of the problems encountered in irregular experiments.

Use of the deviation method requires transcription of a data array presented in terms of plot means to one presented in terms of deviations from either seedlot means or block means. This constitutes an extra step. However, subsequent computations are greatly simplified if the deviations are rounded off to the nearest whole number. Such rounding off is rarely of consequence. I often use deviations in preference to the standard method even when working with regular data arrays.

Adequacy of Deviations when Calculating Sums of Squares

Assume that one wishes to calculate the sum of squares and standard deviation of the series of 10 numbers included under column I in table 1. This can be done in either of two ways:

(1) Square each number as in column II. Find the total of the squares (280). Calculate the total for column I, square it and divide by 10 to obtain the correction factor ($50^2/10 = 250$). The "sum of squares" ($280 - 250 = 30$) is the total of the squares minus the correction factor.

(2) Determine the deviation of each number from the mean of 5.0, enter the deviations in column III, square them (column IV) and sum them to obtain a "sum of squares" of 30.

Notice that the two methods give identical results.

Table 1.--Example showing two ways of calculating the sum of squares of a series of 10 numbers

I Actual	II Square of actual number	III Deviation of actual number from mean	IV Square of deviation
2	4	-3	9
3	9	-2	4
4	16	-1	1
4	16	-1	1
5	25	0	0
5	25	0	0
6	36	+1	1
6	36	+1	1
7	49	+2	4
8	64	+3	9
Total 50	280	0	30
Mean 5.0		0.0	
Correction factor	$50^2/10 = 250$		$0^2/10 = 0$
Sum of squares	30		30

Slight Effects of Small Arithmetic Mistakes or Rounding Off Errors

Assume that one makes a mistake of 1 when adding column I in table 1, obtaining a total of 49 instead of 50. The correction factor would then be calculated as $49^2/10 = 2,401/10 = 240.1$. The sum of squares would be calculated as 280 - 240.1 = 39.9 instead of the true value of 30.

Assume also that a person made the same mistake when working with deviations, obtaining a total of +1 for column III. The correction factor would be calculated as $1^2/10 = .1$. The sum of squares would be calculated as 29.9 instead of 30.

Or, assume that the numbers are changed slightly to produce a total of 49 for column I in table 1. Rounding off the mean of 4.9 to 5.0 and the use of whole-number deviations would result in an error of 0.1 in the sum of squares.

Thus, even with the small deviations shown in table 1, rounding off errors are of little consequence. They are even less important with larger deviations. Also, when using deviations, small arithmetic mistakes may have negligible consequences.

Estimating Seedlot Means by Using Deviations from Block Means

Table 2 is an hypothetical data array showing how to calculate seedlot means by using deviations from block means. It was formulated by assuming a regular experiment with progressively greater growth from block 1 to 5 and from seedlot 1 to 9.

Table 2.--Example showing estimates of seedlot means when calculated from actual values and as deviations from block means for an experiment having many missing plots. (The example was formulated in such a way as to have theoretical means of 0, 7, 8....14 for seedlots 1 to 9, respectively and of 6, 8, 10, 12 and 14 for blocks 1 to 5, respectively, if there were no missing plots.)

Seedlot no.	Actual values: Height in block 1	2	3	4	5	Sum	Mean	Deviations from block means: Height in block 1	2	3	4	5	Sum	Mean	Estimated means: Theory	Act. val.	Deviations
1	2	4	6	--	--	12	4	-4	-4	-4	--	--	-12	-4	6	4	6.1
2	--	--	7	9	11	27	9	--	--	-3	-3	-3	-9	-3	7	9	7.1
3	4	6	8	10	12	40	8	-2	-2	-2	-2	-2	-10	-2	8	8	8.1
4	--	--	9	11	--	20	10	--	--	-1	-1	--	-2	-1	9	10	9.1
5	6	8	10	12	14	50	10	0	0	0	0	0	0	0	10	10	10.1
6	--	--	11	13	--	24	12	--	--	+1	+1	--	+2	+1	11	12	11.1
7	8	10	12	14	16	60	12	+2	+2	+2	+2	+2	+10	+2	12	12	12.1
8	--	--	13	15	17	45	15	--	--	+3	+3	+3	+9	+3	13	15	13.1
9	10	12	14	--	--	36	12	+4	+4	+4	--	--	+12	+4	14	12	14.1
n	5	5	9	7	5	31		5	5	9	7	5	31				
Sum	30	40	90	84	70	314		0	0	0	0	0	0				
Mean	6	8	10	12	14		10.1	0	0	0	0	0		0	10.0	10.1	10.1

The procedure is as follows:

(1) Calculate block totals and means as under "actual values".

(2) Calculate "deviations from block means" as in the central portion of the table.

(3) For each seedlot, add the average "deviation from block mean" to the plantation mean to obtain an estimated true mean (right-hand column).

While there is greater agreement between theory and practice in this hypothetical example than in actual practice, use of the deviations-from-block-means method always gives better estimates of seedlot means than can be obtained by averaging the actual plot means. The larger the number of missing plots and the greater the between-block differences, the greater the advantage of the deviation method.

Analysis of Variance Calculations Using Deviations from Block Means

To compute an analysis of variance, proceed as outlined below and in table 3.

Table 3.--Methods used to calculate means and analysis of variance for an irregular experiment, using deviations from block means (Sums of Squares and Degrees of Freedom are symbolized by SSQ and DF, respectively.)

Seedlot no.	True means for block 1	2	3	4	5	Sum	Ave.	Deviations from block means 1	2	3	4	5	Sum	Freq.	Ave.	Probable true mean
1	A	A	A	-	A			G	G	G	-	G	J	n_s	K	K+E = L
2	A	-	A	A	-			G	-	G	G	-	J	n_s	K	L
3	-	A	A	A	-			-	G	G	G	-	J	n_s	K	L
4	A	A	-	A	A			G	G	-	G	G	J	n_s	K	L
5	A	-	-	A	A			G	-	-	G	G	J	n_s	K	L
6	-	A	A	-	A			-	G	G	-	G	J	n_s	K	L
S	B	B	B	B	B	D		H	H	H	H	H	M		Zero	D
Freq.	n_b	n_b	n_b	n_b	n_b	n_t								n_t		
Mean	C	C	C	C	C		E								Zero	E

G = A - C

$SSQ_{seedlot} = \Sigma(J^2/n_s) - M^2/n_t = \Sigma(J^2/n_s)$, approximately

$SSQ_{block} = \Sigma(B^2/n_b) - D^2/n_t$, approximately

$SSQ_{error} = \Sigma G^2 - \Sigma(n^2/n_b) = \Sigma G^2$, approximately

$DF_{seedlot}$ = number of seedlots - 1

DF_{block} = number of blocks - 1

DF_{total} = total number of plots - 1

Arithmetic checks: each H and M should be less than $\pm n_b/2$ and $n_t/2$, respectively.

(1) For each living plot, enter a true mean (A) as in the left-hand portion of table 3. Calculate the sum, number of plots, and mean for each block (B, n_b and C, respectively) and for the entire plantation (D, n_t and E, respectively).

(2) Prepare a table similar to the right-hand portion of table 3. For each living plot enter a deviation-from-block-mean (G = A - C). Calculate the sum of deviations, number of plots and average deviation for each seedlot (J, n_s and K, respectively) and for the entire plantation (M, n_t and close to zero, respectively). Also, calculate the sum of the deviations (H) for each block.

(3) Check the arithmetic. If there are no mistakes, $\Sigma H = \Sigma J = M$ = less than $n_t/2$; $\Sigma n_b = \Sigma n_s = n_t$; and ΣK = nearly zero. Also, each H and M should be less than $\pm n_b/2$ and $n_t/2$, respectively.

(4) For each seedlot calculate a probable true mean (L = K + E).

(5) Calculate the sums of squares (SSQ) and degrees of freedom (DF) as indicated at the bottom of table 3.

(6) Calculate mean squares (MSQ = SSQ/DF) and F values in the normal manner.

Analysis of Variance Calculations Using Deviations from Seedlot Means

If differences among blocks are small, the analysis of variance can be calculated in terms of deviations from seedlot means, using the methods outlined below:

(1) For each living plot, enter a true mean (A) as in the left-hand portion of table 4. Calculate the sum, number of plots (and mean) for each seedlot (F, n_s and L, respectively) and for the entire plantation (D, n_t and E, respectively).

(2) Prepare a table similar to the right-hand portion of table 4. For each living plot, enter a deviation-from-seedlot-mean. As an arithmetic check, calculate the sum (Q = less than $n_s/2$) for each seedlot.

(3) Calculate the sum of the deviations (P) for each block.

(4) Calculate the sums of squares (SSQ) and degrees of freedom (DF) as indicated at the bottom of table 4.

(5) Calculate mean squares and F values in the normal manner.

When differences among blocks are so small as not to be obvious in the field, they can be ignored in the analysis of variance calculations, often saving one-third in computation time. In this case, the signs of

(3) For each seedlot, add the average "deviation from block mean" to the plantation mean to obtain an estimated true mean (right-hand column).

While there is greater agreement between theory and practice in this hypothetical example than in actual practice, use of the deviations-from-block-means method always gives better estimates of seedlot means than can be obtained by averaging the actual plot means. The larger the number of missing plots and the greater the between-block differences, the greater the advantage of the deviation method.

Analysis of Variance Calculations Using Deviations from Block Means

To compute an analysis of variance, proceed as outlined below and in table 3.

Table 3.--Methods used to calculate means and analysis of variance for an irregular experiment, using deviations from block means (Sums of Squares and Degrees of Freedom are symbolized by SSQ and DF, respectively.)

Seedlot no.	True means for block 1	2	3	4	5	Sum	Ave.	Deviations from block means 1	2	3	4	5	Sum	Freq.	Ave.	Probable true mean
1	A	A	A	-	A			G	G	G	-	G	J	n_s	K	K+E = L
2	A	-	A	A	-			G	-	G	G	-	J	n_s	K	L
3	-	A	A	A	-			-	G	G	G	-	J	n_s	K	L
4	A	A	-	A	A			G	G	-	G	G	J	n_s	K	L
5	A	-	-	A	A			G	-	-	G	G	J	n_s	K	L
6	-	A	A	-	A			-	G	G	-	G	J	n_s	K	L
S	B	B	B	B	B	D		H	H	H	H	H	M		Zero	D
Freq.	n_b	n_b	n_b	n_b	n_b	n_t								n_t		
Mean	C	C	C	C	C		E								Zero	E

$G = A - C$

$SSQ_{seedlot} = \Sigma(J^2/n_s) - M^2/n_t = \Sigma(J^2/n_s)$, approximately

$SSQ_{block} = \Sigma(B^2/n_b) - D^2/n_t$, approximately

$SSQ_{error} = \Sigma G^2 - \Sigma(n^2/n_b) = \Sigma G^2$, approximately

$DF_{seedlot}$ = number of seedlots - 1

DF_{block} = number of blocks - 1

DF_{total} = total number of plots - 1

Arithmetic checks: each H and M should be less than $\pm n_b/2$ and $n_t/2$, respectively.

(1) For each living plot, enter a true mean (A) as in the left-hand portion of table 3. Calculate the sum, number of plots, and mean for each block (B, n_b and C, respectively) and for the entire plantation (D, n_t and E, respectively).

(2) Prepare a table similar to the right-hand portion of table 3. For each living plot enter a deviation-from-block-mean (G = A - C). Calculate the sum of deviations, number of plots and average deviation for each seedlot (J, n_s and K, respectively) and for the entire plantation (M, n_t and close to zero, respectively). Also, calculate the sum of the deviations (H) for each block.

(3) Check the arithmetic. If there are no mistakes, $\Sigma H = \Sigma J = M$ = less than $n_t/2$; $\Sigma n_b = \Sigma n_s = n_t$; and ΣK = nearly zero. Also, each H and M should be less than $\pm n_b/2$ and $n_t/2$, respectively.

(4) For each seedlot calculate a probable true mean (L = K + E).

(5) Calculate the sums of squares (SSQ) and degrees of freedom (DF) as indicated at the bottom of table 3.

(6) Calculate mean squares (MSQ = SSQ/DF) and F values in the normal manner.

Analysis of Variance Calculations Using Deviations from Seedlot Means

If differences among blocks are small, the analysis of variance can be calculated in terms of deviations from seedlot means, using the methods outlined below:

(1) For each living plot, enter a true mean (A) as in the left-hand portion of table 4. Calculate the sum, number of plots (and mean) for each seedlot (F, n_s and L, respectively) and for the entire plantation (D, n_t and E, respectively).

(2) Prepare a table similar to the right-hand portion of table 4. For each living plot, enter a deviation-from-seedlot-mean. As an arithmetic check, calculate the sum (Q = less than $n_s/2$) for each seedlot.

(3) Calculate the sum of the deviations (P) for each block.

(4) Calculate the sums of squares (SSQ) and degrees of freedom (DF) as indicated at the bottom of table 4.

(5) Calculate mean squares and F values in the normal manner.

When differences among blocks are so small as not to be obvious in the field, they can be ignored in the analysis of variance calculations, often saving one-third in computation time. In this case, the signs of

the deviations can be ignored and the P's need not be calculated. When the F ratio for block is less than 3.0, separation of the block from the error sum of squares usually has a negligible effect on the error term.

Table 4.--Methods used to calculate means and analysis of variance for an irregular experiment, using deviations from seedlot means. (Sums of Squares and Degrees of Freedom are symbolized by SSQ and DF, respectively. Except where otherwise noted, symbolism is the same as for table 3.)

Seedlot no.	True means for block								Deviations from seedlot means						Deviation of true sum from expected sum
	1	2	3	4	5	Sum	Freq.	Ave.	1	2	3	4	5	Sum	
1	A	A	A	-	A	F	n_s	L	N	N	N	-	N	Q	$J = F - n_s E$
2	A	-	A	A	-	F	n_s	L	N	-	N	N	-	Q	J
3	-	A	A	A	-	F	n_s	L	-	N	N	N	-	Q	J
4	A	A	-	A	A	F	n_s	L	N	N	-	N	N	Q	J
5	A	-	-	A	A	F	n_s	L	N	-	-	N	N	Q	J
6	-	A	A	-	A	F	n_s	L	-	N	N	-	N	Q	J
Sum						D			P	P	P	P	P	M	
Freq.							n_t		n_b	n_b	n_b	n_b	n_b	n_t	
Mean								E							Zero

$N = A - L$

$SSQ_{seedlot} = \Sigma(J^2/n_s)$, approximately

$SSQ_{block} = \Sigma(P^2/n_b)$, approximately

$SSQ_{error + block} = \Sigma N^2$, approximately

$SSQ_{error} = SSQ_{error + block} - SSQ_{block}$

$DF_{seedlot}$, DF_{block}, DF_{total} = number of seedlots - 1, number of blocks - 1, and total number of plots - 1, respectively.

Arithmetic checks: each Q and M should be less than $\pm n_s/2$ and $n_t/2$, respectively.

Analysis of Variance, Using Data from Several Plantations

The procedures when using data from several plantations are described below and in table 5:

(1) Start with single-plantation analyses as outlined in tables 3 or 4.

It is not necessary to use the same calculation method for all plantations.

Table 5.--Methods used to calculate means and analysis of variance for an irregular experiment involving several plantations, using deviations from plantation means as calculated by use of tables 3 or 4 (Symbolism is a continuation of that used in those tables.)

Seedlot no.	Sum of deviations: Plantation 1	2	3	4	Sum	Number of plots: Plantation 1	2	3	4	Sum	Average deviation	Probable true mean
1	J	J	-	J	R	n_s	n_s	-	n_s	n_{sp}	$S = R/n_{sp}$	$U = S + T$
2	J	-	J	J	R	n_s	-	n_s	n_s	n_{sp}	S	U
3	J	-	-	J	R	n_s	-	-	n_s	n_{sp}	S	U
4	J	J	J	-	R	n_s	n_s	n_s	-	n_{sp}	S	U
5	J	J	J	J	R	n_s	n_s	n_s	n_s	n_{sp}	S	U
6	J	J	-	-	R	n_s	n_s	-	-	n_{sp}	S	U
Sum	Nearly Zero					n_t	n_t	n_t	n_t	n_{gt}		
Mean											Zero	T
	ACTUAL SUMS AND MEANS											
Sum	D	D	D	D	V							
Mean	E	E	E	E								$T = V/n_{gt}$

$SSQ_{seedlot} = \Sigma(R^2/n_{sp})$

$SSQ_{seedlot + seedlot\ X\ plantation} = \Sigma(J^2/n_s)$

$SSQ_{seedlot\ X\ plantation}$ is obtained by subtraction.

$SSQ_{plantation} = \Sigma(D^2/n_t) - V^2/n_{gt}$

$SSQ_{block\text{-}within\text{-}plantation}$ and SSQ_{error} are obtained by adding the SSQ_{block} and SSQ_{error}, respectively for the individual plantations.

$DF_{seedlot}$ and $DF_{plantation}$ are the number of seedlots - 1, and the number of plantations - 1, respectively.

$DF_{seedlot + seedlot\ X\ plantation}$ = the sum of the $DF_{seedlot}$ for the individual plantations; obtain $DF_{seedlot\ X\ plantation}$ by subtraction.

DF_{block} and DF_{error} = the sums of these for individual plantations.

If the measurements and analysis have been done by different individuals and in different units, a simple conversion is possible. If, for example, some plantations were measured in inches and others in centimeters, and all are to be converted to centimeters, all means and deviations based on inches should be multiplied by 2.54 and all sums of squares or mean squares based on inches should be multipled by $2.54^2 = 6.45$.

(2) Prepare a table similar to the left-hand portion of table 5 and insert a plantation-sum-of-deviations (J) and the number of plots (n_s) for each seedlot represented in any one plantation. For each plantation, insert the actual sum (D), actual mean (E) and total number of plots (n_t).

(3) For each seedlot, calculate the total sum-of-deviations (R) and average deviation ($S = R/n_{sp}$). For all plantations combined, calculate the actual sum (V), number of plots (n_{gt}) and mean (T).

(4) Calculate the probable true mean ($U = S + T$) for each seedlot.

(5) Calculate the sums of squares as indicated at the bottom of table 5. The sums of squares for error, block-within-plantation and "seedlot + seedlot X plantation" have already been calculated for individual plantations and may be summed to obtain the values for all plantations combined.

(6) Calculate degrees of freedom as indicated.

(7) Compute mean squares and F values in the normal manner.

LEAST SQUARES TECHNIQUE FOR USE WITH COMPUTERS

A least squares technique is available for those with access to a computer. This technique can be used with data such as are considered here. The analysis procedures are such as to stretch the capacity of even the largest computers. The routines are therefore effectively limited to experiments including a few score seedlots and a few plantations.

LIMITATIONS AND ADVANTAGES OF THE DEVIATIONS TECHNIQUES

With perfectly regular experiments, the deviations-from-means techniques yield exactly the same results as would be obtained with normal calculation procedures.

The standard method for calculating missing-plot values is laborious and therefore effectively limited to experiments with less than a few dozen missing plots. The simple substitution methods (seedlot mean or randomly chosen plot mean) are limited to experiments with small between-block differences and less than 10 percent missing plots. There are no such limitations to the use of the deviations-from-means techniques.

They can yield satisfactory results even if 50 percent of the plots are missing and there are large differences among blocks.

Whatever the calculation method, seedlots represented by a single plot in one plantation or in one plantation only in the case of a multi-plantation experiment should be excluded from an analysis of variance. Otherwise, the sum of squares due to seedlot will be inflated.

One situation is not amenable to the techniques described here: that in which certain blocks or plantations are composed primarily of seedlots considerably above or below average in the trait being measured. This might happen if seedlots of supposedly rapid growth are assigned to one series of plantations and seedlots of supposedly slow growth are assigned to other plantations. In such a case, there is a confounding of between-seedlot and between-block or between-plantation differences such that none of them can be estimated accurately.

Although the unequal sample sizes found in an irregular experiment do not pose serious problems in the calculation of analysis of variance or probable true means, they do in estimating the significance of a difference between any two means. Suppose that some seedlots are represented in 10 blocks and others in only 5 blocks. "Least Significant Differences" or multiple-range tests applicable to the former are not applicable to the latter. In such a case, means must be compared individually.

The deviations-from-mean techniques saves the most time in large experiments containing 100+ seedlots and several plantations, none of them complete. In such a case, the only reasonable alternative may be the exclusion of several seedlots or plantations from the analysis.

Even if few plots are missing, working with deviations can save time because of the lesser need for absolute accuracy in the computation work. As explained in connection with table 1, the smallest arithmetic mistake may have large consequences when working with true means and their sums of squares. That being the case, it is necessary to check every arithmetic operation until the slightest discrepancies are eliminated. Frequently, that requires much time. If working with deviations and their sums of squares, many slight arithmetic mistakes can be forgiven, so that much less cross checking is necessary.

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VARIATION IN MONOTERPENE CONTENT AMONG GEOGRAPHIC SOURCES OF EASTERN WHITE PINE[1]

A. R. Gilmore and J. J. Jokela

ABSTRACT.--Variations of monoterpenes in cortical oleoresins and foliar samples were determined for seed from 16 provenances of eastern white pine (Pinus strobus L.). The experiment was analyzed using the "raw" and the arcsine "transformed" data. Alpha-pinene, camphene, and β-pinene varied between seed sources when "raw" data were analyzed and α-pinine, β-pinene, and myrcene varied between seed sources when "transformed" data were analyzed. No trend was detected for latitude or longitude of seed origin for any monoterpene. Foliar monoterpenes did not vary among seed sources and no geographic pattern or trend was indicated for any monoterpene.

Previous studies have shown regional variation in the monoterpenes of certain coniferous species. For example, Gilmore (1971) found that α-pinene in loblolly pine increased almost linearly from the southernmost to northernmost seed source; Smith (1977) reported evidence to support the establishment of at least five regions and four transition-zone types of ponderosa pine; Hanover (1966) showed most of the variations in monoterpene content in western white pine to be genetically controlled; and Hilton (1968) found that the concentration of five monoterpenes in 23 geographic sources of eastern white pine varies, but he could not show distinct geographic patterns for these monoterpenes.

A range-wide provenance test of 16 sources of eastern white pine (Pinus strobus L.) planted in west central Illinois in 1959 provided an opportunity to study the relation between monoterpenes and

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geographic origin of white pine growing on a uniform site outside its natural range. The most southerly natural occurrence of white pine in the Mississippi River Valley is in northern Illinois, which is about 100 miles north of the plantation used in this study.

METHODS

Seed from 16 provenances of eastern white pine were collected by the U.S. Forest Service (table 1) and sown during the 1957 and 1958 growing seasons in the State Tree Nursery at Morgantown, North Carolina.

Table 1.--Origin of eastern white pine provenances

Location	North latitude	West longitude	Elevation in feet
Union County, Georgia (GA)	34°46'	84°03'	2450
Greene County, Tennessee (TN)	36°00'	82°48'	2250
Pulaski County, Virginia (VA)	37°05'	80°50'	2400
Monroe County, Pennsylvania (PA)	41°05'	75°25'	1800
Franklin County, New York (NY)	44°25'	74°15'	1600
Penobscot County, Maine (ME)	44°51'	68°38'	150
Ashland County, Ohio (OH)	40°45'	82°15'	1000
Allamakee County, Iowa (IA)	43°28'	91°30'	1000
Cass County, Minnesota (MN)	47°23'	94°25'	1300
Forest County, Wisconsin (WI)	45°51'	88°54'	1500
Newaygo County, Michigan (MI)	43°30'	85°40'	600
Algoma District, Ontario (ON)	46°10'	82°37'	650
Pontiac County, Quebec (PQ)	47°30'	77°00'	1000
Lunenburg County, Nova Scotia (NS)	44°25'	64°35'	150
Transylvania County, North Carolina (NC)	35°14'	82°38'	2120
Greenbrier County, West Virginia (WV)	38°02'	80°30'	2600

In 1959, 2-0 seedlings of all provenances except Michigan were planted in 4-tree row plots, replicated 12 times. Seedlings from the Michigan source were planted in 1961 as 2-1 stock. The experimental trees were spaced 7 feet apart, in rows 14 feet apart. White pine seedlings planted in intervening "filler" rows were removed after the 1970 growing season. The plantation is located in the "sand hill" area of west central Illinois in Cass County. The soil type is a Plainfield fine sand (Typic Udipsamments) and is fairly uniform over the planted area. Growth and survival data have been obtained periodically; the last measurements were taken in the fall of 1973. Survival at the end of the first growing season ranged from 52 to 90 percent. Fail spots were replanted in 1961 with surplus stock (2-2) that had been grown an additional 2 years in the Mason State Tree Nursery in Illinois. Survival of provenances after the 1973 growing season ranged from 90 to 100 percent and averaged 97 percent.

The plantation was thinned to two trees per plot in December, 1974, when it was 16 years old. The uncut trees represented the best of the largest trees. Before the plantation was thinned, a cut was made through the bark at breast height on 8 to 10 sample trees per provenance. The oleoresin exuding from each cut was collected in a small vial, which was placed on ice for transport to the laboratory where the sample was frozen until it was analyzed.

Foliar samples were taken from the upper part of the crown of each sample tree when felled. The needles were placed in plastic bags in an ice cooler for transport to the laboratory where they were frozen until analyzed.

Cortical oleoresin samples were dissolved in hexane containing 670 ppm cumene and this solution was analyzed for monoterpenes. Each monoterpene was computed as percentage of weight of the oleoresin sample, using cumene as the internal standard.

Monoterpenes in the foliar samples were determined by injecting a solid segment of needle without cumene into the gas chromatograph, as described by Roberts (1968). The content of each foliar monoterpene was expressed as a percentage of the total monoterpene concentration in the sample. Otherwise, analytical procedures were the same as those for the cortical oleoresin samples.

Samples were analyzed with a Hewlett-Packard Model 5750 Gas Chromatograph equipped with dual hydrogen-air ionization detectors using stainless steel columns with 20 percent carbowax 20M liquid phase on 60-80 mesh, acid-washed chromosorb W solid support. Operating conditions were: injection port, 200°C; detector 225°C; column 110°C; and He flow 17 cm^3/min.

The raw data were statistically analyzed as a complete randomized design, and differences between provenances for each significant variable were determined by the least-significant-difference method. In addition, the raw data of the monoterpenes in the cortical oleoresin samples were transformed using an arcsine function and analyzed as were the raw data.

RESULTS AND DISCUSSION

The average height and diameter ($d_1b_1h_1$) of sample trees according to seed source are shown in Table 2. The average height of all sample trees was equal to the plantation average (26 feet), whereas the diameter of cut trees was slightly smaller than the plantation average - 5.0 vs. 5.3 inches. Differences in average height or average diameter between sample and all trees in a seed source was small, never exceeding 2 feet in height or 0.6 inches in diameter. Therefore, the sample trees were considered to be reasonably representative of the plantation.

Table 2.--Average heights and diameters (d.b.h.) of sample trees at end of 1973 growing season by seed source

Seed source	Height[1/]	Seed source	Diameter[1/]
	feet		inches
GA	31	TN	6.4
TN	29	GA	6.2
NC	29	PA	5.8
PA	29	OH	5.7
WI	27	NC	5.6
MI	27	MI	5.5
OH	27	WI	5.2
NS	26	WV	5.0
WV	26	NS	4.9
MN	25	MN	4.9
ON	25	VA	4.5
NY	25	PQ	4.5
VA	24	ON	4.5
PQ	23	NY	4.3
IA	20	ME	3.6
ME	19	IA	3.6
Average	26	Average	5.0

1/ Any two averages not included within the same line appearing to the right of each ranking of source are significantly different at 5-percent level.

Six monoterpenes were found in the cortical oleoresin of all seed sources. The order in which they eluted from the gas chromatographic column were α-pinene, camphene, β-pinene, myrcene, limonene, and β-phelledrene. Hilton (1968) reported two additional monoterpenes in eastern white pine than were reported in this study: 3-carene and terpinolene. This discrepancy can be explained by the fact that 3-carene was eluting from the gas chromatographic column at the same time as our marker cumene and could not be detected. This resulted in the amount of cumene appearing greater under the curve on the chromatogram chart than its actual amount. Therefore, an error might have been introduced in computing the quantities of monoterpenes in a sample. But when we determined the monoterpenes in a number of our cortical oleoresin samples without using cumene, we found that the concentrations of 3-carene was low when compared to the other monoterpenes in the sample and in some samples it was not detected. So, we feel confident that our conclusions from this study are valid and would not change, even if some other marker was used that would not interfere with the

monoterpenes eluting from the gas chromatograph. We did not report terpinolene in the study as it was detected in only a few samples and the incidence was too small and varied among seed source to be considered.

The composition of those monoterpenes in cortical oleoresins that varied significantly among seed sources and the ranking of these sources is shown in Table 3. Although significant differences were found between provenances for α-pinene, camphene, and β-pinene when the raw data were analyzed, no distinct geographic concentration pattern was established because no significant correlation was found between latitude or longitude of the seed source and any of the monoterpenes in the oleoresin. These results agree with Hilton (1968) who did not establish a monoterpene pattern for eastern white pine. But it is interesting to note that the New York seed source had the highest concentration of camphene and β-pinene and the next to highest concentration of α-pinene. On the other hand, the Ohio seed source had the lowest concentration of camphene and β-pinene but was average in its concentration of α-pinene. The raw data averages of the three monoterpenes that did not differ among seed source were: myrcene, 2.5; limonene, 0.1; and β-phelledrene, 0.8.

When the raw data of cortical oleoresin was converted using arcsine transformations, the analyses showed that α-pinene, β-pinene, and myrcene varied significantly among seed sources at the 5-percent level, whereas camphene differences only approached significance at this level (table 4). But the relative rank of seed sources for each monoterpene was almost the same regardless of the method of anlaysis used (raw data or transormation).

There were some tree-to-tree variations in the composition of the monoterpene fractions in the cortical oleoresin, not only for all trees but also for trees within a seed source. Similar results have been shown by numerous investigators for other tree species.

The average concentrations and range of monoterpenes in the foliage are shown in table 5. Foliar monoterpenes varied widely among trees and there was no significant difference between seed sources and no geographic concentration pattern or trend was indicated. It would have been interesting from an academic viewpoint to determine the chemical composition of the four unknown monoterpenes. But because of the findings in this phase of the study, the time and expense could not be justified at this time.

CONCLUSION

Although we found distinct differences in some cortical monoterpenes between provenances, white pine does not seem to exhibit a distinct geographic concentration pattern for any of the detected monoterpenes. As shown in this and other studies, cortical and foliar monoterpenes vary widely between trees within a seed source.

Table 3.--Ranking of white pine sources by average percentages of raw data of α-pinene, camphene, and β-pinene in cortical oleoresin samples[1/]

α-pinene		Camphene		β-pinene	
Source	Oleoresin	Source	Oleoresin	Source	Oleoresin
	Percent		Percent		Percent
PA	8.11	NY	1.28	NY	8.41
NY	7.09	ME	1.20	WV	6.96
WV	6.36	WV	1.11	MN	6.70
ME	6.25	PA	1.09	ON	6.68
PQ	5,83	GA	1.03	NC	6.12
MN	5.70	IA	0.94	IA	6.09
OH	5.64	PQ	0.93	VA	5.95
TN	5.60	MN	0.92	PQ	5.93
GA	5.38	NC	0.91	TN	5.88
ON	5.29	TN	0.87	GA	5.59
NC	4.80	ON	0.85	WI	5.37
NS	4.61	MI	0.85	MI	4.97
WI	4.55	WI	0.73	ME	4.92
IA	4.47	NS	0.66	PA	4.69
VA	4.22	VA	0.60	NS	4.57
MI	3.98	OH	0.60	OH	1.88
Average	5.49	Average	0.91	Average	5.66

1/ Any two averages not included within the same line appearing to the right of each ranking of source are significantly different at 5-percent level.

Table 4.--Ranking of white pine sources by average percentages based on transformed data of α-pinene, β-pinene, and myrcene in cortical oleoresin samples [1/]

α-pinene		β-pinene		Myrcene	
Source	Oleoresin	Source	Oleoresin	Source	Oleoresin
	Percent		Percent		Percent
PA	7.7	NY	7.8	OH	7.6
NY	7.0	MN	6.5	PA	6.2
ME	6.0	WV	6.3	WV	5.8
WV	5.9	IA	5.9	MI	2.8
PQ	5.7	NC	5.8	NS	2.9
MN	5.7	TN	5.7	NC	2.5
OH	5.6	PQ	5.7	TN	2.0
TN	5.5	VA	5.6	VA	1.7
GA	5.1	GA	5.3	WI	1.8
ON	5.1	ON	5.2	IA	1.6
NC	4.6	WI	4.9	ME	1.6
WI	4.4	MI	4.8	PQ	1.2
NS	4.4	ME	4.7	NY	1.2
IA	4.4	PA	4.3	GA	0.6
VA	4.1	NS	4.1	MN	0.6
MI	3.9	OH	1.7	ON	0.4
Average	5.3	Average	5.3	Average	5.7

1/ Any two averages not included within the same line appearing to the right of each ranking of source are significantly different at 5-percent level.

Table 5.--Monoterpene content of foliar oleoresin for 16 geographic sources of eastern white pine (raw data)

(In percent)

Terpene	Range of source means	Average of source means
α-pinene	4.7 - 10.3	6.8
Camphene	53.6 - 65.6	59.0
β-pinene	5.0 - 18.0	12.4
Myrcene	16.6 - 23.3	19.4
Limonene	5.2 - 13.3	8.7
Unknown 1	1.0 - 10.7	5.3
Unknown 2	1.0 - 3.5	1.8
Unknown 3	1.0 - 7.0	2.1
Unknown 4	3.4 - 8.7	5.5

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THE YELLOW X PAPER BIRCH HYBRID--A POTENTIAL SUBSTITUTE FOR YELLOW BIRCH ON PROBLEM SITES

Knud E. Clausen[1]

ABSTRACT--Yellow x paper birch hybrids and yellow birches with common female parents were compared after 5 growing seasons in an open field. Survival of the hybrids was 91 percent compared with 64 percent for the yellow birch trees. The hybrids were from 25 to 32 percent taller than the yellow birches and had 19-40 percent greater diameter. Because this hybrid not only grows better than yellow birch but also withstands greater environmental stress, it appears suitable for planting on open or partially shaded sites where ecological conditions are less favorable for yellow birch.

Natural interspecific hybridization is common in the genus Betula but natural hybrids between yellow birch (B. alleghaniensis Britt.) and paper birch (B. papyrifera Marsh.) have only been reported from Iowa, Minnesota, Wisconsin, Michigan, and New Hampshire (Clausen 1973b, Barnes et al. 1974). However, hybridization between these species apparently does occur over much of the range of yellow birch. When we grew yellow birch seedlings for a range-wide provenance study, yellow x paper birch hybrids appeared in 32 out of 55 seed sources. The sources with hybrids came from 13 states and 4 Canadian provinces ranging from Newfoundland to Minnesota and from Quebec to Georgia (Clausen 1973b). Because the hybrid seedlings grew faster than the yellow birch seedlings in the nursery, I decided to test this hybrid against yellow birch and to try to determine its potential as a possible substitute for yellow birch on certain sites.

METHODS

In the spring of 1972, when a yellow birch progeny test was

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established near Lake Tomahawk in Oneida County, northcentral Wisconsin, a number of yellow x paper birch hybrids were included. The seedlings for the progeny test were grown in a nursery at Rhinelander, Wisconsin, as previously described (Clausen 1973a), and planted in the field when 4 years old. The test site, a former potato field and pasture on Padus sandy loam, was kept clean-cultivated through the summer of 1975 and later mowed. The plantation included 144 yellow birch families in a split-plot design with 6 or 8 replications and hybrid trees of 3 families in 1, 5, and 12 replications, respectively. All families were set out in 4-tree plots and at 8 x 8 ft. spacing. From 1 to 3 hybrid trees of 13 additional families were planted in the border rows of the plantation. Two of these families were from Canadian sources that had not produced hybrids when used in the provenance test.

Height and diameter at one-half tree height were measured after 5 growing seasons in the field. This paper is mainly concerned with those hybrid and yellow birch groups that had female parents in common and were growing together in the same replications. Because suitable yellow birch comparison plots were not available for about one-half of the hybrid plots and 13 of the hybrid families, these plots were not used in the analyses but did provide supporting information.

RESULTS

Previous publications reported the performance of the yellow birch families included in the progeny test after 4 years in the nursery (Clausen 1973a) and after 3 years in the field (Clausen 1975) but did not include any information about the performance of the hybrids. By the same token, the major portion of the 5-year yellow birch data will be presented elsewhere.

All trees in the hybrid plots with useable comparison plots were alive at the end of the fifth growing season in the field and only two comparison trees in one yellow birch family had died. Survival of all hybrids in the plantation was 91 percent compared with 64 percent for the yellow birch trees used as comparison trees or growing adjacent to the hybrids. Thus, the yellow x paper birch appears to survive better than yellow birch when planted under open field conditions.

Most hybrids followed the trend observed earlier in the nursery, and were still growing faster than yellow birch after 5 years in the field. The yellow birch comparison families averaged 174 cm in height compared with 221 cm for the hybrids (table 1). Of the individual families, hybrid group 2964-5, on the average, was 25 percent taller than its comparison group. A t-test showed that this difference was significant at the 0.5 level. The superiority in individual blocks ranged from 15 to 34 percent. Similarly, the superiority of the hybrids over the related yellow birches in family 2983-1 ranged from 8 to 60

percent and averaged 28 percent. This difference was not significant according to the t-test, probably because the differences between hybrids and yellow birch were small in 2 of the 4 blocks. The single plot of hybrid family 2983-10 was 32 percent taller than the yellow birch plot (table 1).

Table 1.--Mean height and diameter of yellow birch and yellow x paper birch hybrids after 5 years in the field

Family	Type of birch	No. of plots	Mean height	Percent superiority Mean	Percent superiority Range	Mean diameter 1/	Percent superiority Mean	Percent superiority Range
			cm			mm		
2964-5	Yellow	4	172	--	--	13.6	--	--
	Hybrid	4	214*	25	15-34	16.1	19	4-36
2983-1	Yellow	4	176	--	--	13.1	--	--
	Hybrid	4	226	28	8-60	16.2*	24	3-31
2983-10	Yellow	1	174	--	--	11.4	--	--
	Hybrid	1	230	32	--	15.9	40	--
Means:	Yellow birch		174			13.1		
Means:	Hybrid		221			16.1		

1/ Measured at one-half tree height.
* Difference between yellow birch and hybrid is significant at .05 level (t-test).

When additional hybrid plots of families 2964-5 and 2983-1 and of 12 other families were compared with unrelated yellow birches in adjacent plots, the hybrids were taller in 18 plots, of equal size in 1 plot, and smaller than yellow birch in 5 plots. So in general, the yellow x paper birch hybrid does have faster height growth than yellow birch does.

Tree diameter followed a pattern similar to that for tree height. The yellow birch families in the 9 comparison plots averaged 13.1 mm in diameter at one-half tree height as against 16 mm for the hybrids (table 1). Hybrid group 2983-1 had a significantly greater diameter than its comparison trees with an average superiority of 24 percent and a range between blocks from 3 to 31 percent. The diameter of hybrid plot 2983-10 was 40 percent greater than that of the yellow birch comparison plot. In the comparisons between hybrid plots and unrelated yellow birches, the hybrids had greater diameter in 21 plots, were of equal size in 1

plot, and had smaller diameter than yellow birch in 2 plots. Thus, the hybrid also has better diameter growth than yellow birch in most cases.

The superiority of the hybrid over yellow birch was also evident when single trees were compared. The tallest tree in a hybrid plot (family 2964-5) was 330 cm while the tallest yellow birch in a comparison plot (family 2983-1) measured 280 cm. This is a difference of 18 percent. In addition, the tallest tree per plot exceeded 200 cm in all of the 15 surviving hybrid groups, whereas only 5 of the yellow birch families had trees 200 cm and above. Although the greatest mid-tree diameter observed, 25 mm, was the same for both the hybrid and for yellow birch, nine hybrid groups had trees with diameters 15 mm and above, while only four yellow birch families had trees in this class.

DISCUSSION

The fact that hybrid seed is produced by yellow birch parent trees over much of the range of yellow birch (Clausen 1973b), indicates that hybridization between yellow birch and paper birch can take place wherever the two species occur together. The relative scarcity of reported hybrids may mean that the hybrids have difficulty getting established under natural conditions due to lack of suitable seedbeds and to competition from other plants. The 20 hybrids located by Barnes et al. (1974) were all growing in disturbed habitats which would have had exposed mineral soil, abundant light and little competition at the time of establishment. These authors also suggested that the hybrids were most likely to be found on mesic or wet-mesic sites. This is not too surprising because most hybrids have been found growing in association with yellow birch which typically occurs on such sites. However, the results of this study show that the hybrid can survive and grow very well on a much drier site. One could, in fact, expect such a possibility because the other parent of the hybrid, paper birch, is a pioneer species capable of growing on a wide range of sites, including fairly dry ones (Fowells 1965).

Open-field sites such as the one used in this test may not provide the best conditions for the growing of yellow birch but apparently are well suited for the hybrid. On such sites, control of competing vegetation is fairly easily accomplished and undoubtedly benefits both species. The abundance of light on open field sites appears to favor the hybrid, whereas our experience indicates that yellow birch will grow better under some degree of shade. The hybrid will also withstand greater environmental stress than yellow birch. Many trees in this plantation were left in a weakened condition at the end of the 1976 growing season due to a severe drought during the summer and fall. A light snow cover over the winter followed by hot, dry weather during May 1977 lead to further tree mortality so that only 78 percent of the hybrids and 35 percent of the yellow birches used in the comparisons were alive in June of that year.

Although the natural hybrid has shown better growth than yellow birch, it may not be superior to its other parent, paper birch. There were no paper birch trees included in this plantation so a direct comparison is not possible in this case. However, hybrids from controlled crosses between the two species have been grown and compared with trees of both parent species. In those tests, the hybrids again were faster growing than yellow birches of the same age, but were either slower growing or, at best, grew as fast as paper birch (Clausen 1973b).

The superior growth of the yellow x paper birch hybrid and its greater ability to survive in the open suggests to me that this hybrid would make a good tree to plant in places where the ecological conditions are less favorable for yellow birch. In other words, the hybrid could be planted in open or partially shaded areas where it could take advantage of the high degree of insolation and still would be able to tolerate the drier site. So far we don't know what the wood characteristics of the hybrid are but assuming that they are similar to those of yellow birch, the proposed use of the hybrid would enable us to grow a tree resembling yellow birch on a wider range of sites.

How then can one obtain planting stock of this hybrid? Although it is not difficult to recognize the hybrid in nursery beds of yellow birch, this method is impractical because less than 1 percent and rarely as much as 10 percent of the seedlings are usually hybrids (Clausen 1973b). A much more efficient way is to use controlled pollination. This is most easily carried out on grafted trees in the greenhouse but can also be done in the field. Yellow birch females should be pollinated with paper birch pollen because the chance of success and the yield of good seed is poorer when the cross is made in the opposite direction (Clausen 1970). Seed yield from yellow x paper birch crosses is about as good as that of open-pollinated yellow birch where it typically ranges from 150 to 200 seeds per catkin or occasionally higher. The hybrid seed tends to be of pooer quality than that of yellow birch but usually averaged about 50 percent germination. Because even a small yellow birch graft can have 10 to 20 female catkins, it is thus possible to produce a large amount of hybrid seed with relatively little effort.

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